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THE RELATION BETWEEN TEMPERATURE AND THE PEDAL RHYTHM OF BALANUS.

By WILLIAM H. COLE.

(From the Physiological Laboratory of Rutgers University, New Brunswick.)

(Accepted for publication, January 28, 1929.)

The characteristic contractions and expansions of the pedal cirri,—a respiratory and feeding mechanism,—in the rock barnacle, *Balanus balanoides* L., have proved to be reliable indicators of stimulation. The animal as a whole, and the cirri alone, are very sensitive to chemical, mechanical, photic, and thermal stimuli, the effects of which may be measured by observing the corresponding changes in the character and rate of the pedal rhythm. It is possible therefore to investigate such stimuli qualitatively and in some cases quantitatively, either separately or in appropriate combination, while the others are so far as possible kept constant. A preliminary study of this sort was recently made[1] on one group of *B. balanoides*. The relations between temperature and changes in the pedal rhythm are described in this paper.

Consistent results from changes of temperature are obtained only when the other external conditions are kept as constant as possible. The effects of various external factors, as well as of internal conditions, if not controlled, would lead to an erroneous conclusion concerning the behavior of the barnacle. Here is an animal activity influenced by several factors, but it would be absurd to conclude that their effects cannot be studied separately. If the various factors can be determined and controlled the analysis of any one becomes possible. Merely because an activity is "complex" is not sufficient reason for abandoning efforts to analyze it (*cf.* Herber and Slifer, 1928). In the laboratory regular movements of the cirri demand running water, with a rate of flow within definite limits. This was obtained by means

[1] The experiments were done during the summer of 1928 at the Mount Desert Island Biological Laboratory, where Director Dr. H. V. Neal extended many appreciated courtesies.

of a water gauge which registered the flow in cubic centimeters per minute within an error of 2 per cent. To prevent as much as possible contact stimulation by particles in the water without decreasing the food supply, the water was filtered through cloth with a 1 mm. mesh. Contact stimuli due to feces were rare since the latter sank upon extrusion. Vibrational stimuli were decreased to a minimum by mounting the aquarium on rubber balls set on a layer of moderately soft sheet rubber 15 mm. thick. Illumination was furnished by northern sky light or by Mazda lamps the intensity of which did not change enough during an experiment to elicit the well known shading reflex. To avoid effects due to changes in the food supply or to the physical constants of the water such as pH, specific gravity and salinity, the experiments were done only when any variations in those factors were physiologically insignificant. Interfering stimuli caused by moulting or defecation were eliminated from the results by omitting observations during and immediately following those activities.

A group of 41 animals attached to a stone was placed in a glass aquarium of 650 cc. capacity. The water entered at the bottom at the rate of 250 cc. per minute and overflowed around the top. The resultant immersion of these intertidal animals, continuous for 9 weeks, had no ill effect that could be determined. The temperature of the water was controlled by sending the current through a lead coil (1.5 m. long) immersed in a 12 liter water bath, to which ice, or cold or hot water was added as desired. The temperature of the aquarium water was changed between 0.0° and 28.0°C. either upward or downward by not more than 3° at a time, and not oftener than once in 40 minutes. Allowing 15 minutes for equilibrium the temperature remained constant to within 0.1° for periods of from 15 to 60 minutes, depending upon the ratio of the temperatures of the air and the bath. At each temperature the time required for 10 contractions and expansions was measured by a stop-watch to 0.05 second. The averages of 5 such readings were found to be significant because of the remarkable regularity of the movement under constant conditions. Complete records of 17 individuals, the basal diameters of which varied from 4 to 15 mm., were secured over a period of 9 weeks, involving 965 averages of 6,775 observations at 186 different temperatures between 2.0° and 27.0°C. Twelve hundred observations on 10 other animals

showed similar results but are not included in this report because of their incompleteness over an adequate range of temperatures.

In general, the rate of the pedal rhythm of the barnacle increased regularly with temperature from 2.0° to 21.0°C., revealing a critical temperature above which the increment was slightly decreased. Below 2.0° the movements were extremely irregular and often spasmodic. Between 21.0° and 27.0° three different effects appeared in different cases: (1) the rate decreased or became irregular, (2) the rate continued to increase, or (3) the valves closed. Beyond 27.0° closure of the valves regularly occurred. In the case of the few animals whose rates continued to increase beyond 21.0° closure at 27° was sudden and not preceded by irregularity. Alteration of the rate by temperature was due primarily to a change in the duration of the expanded phase, without much change in the contraction period, a fact easily demonstrated at the lower temperatures. It may be said therefore that temperature effects in the barnacle are primarily "diastolic."

In any one animal the frequency of movement at a given temperature on different days varied only about 5 per cent. Since this latitude of variation was constant throughout the temperature range, data obtained over a long period were combined. Having once determined the relation between frequency and temperature it was possible to predict the temperature of the water to within 1.0° from observations on the rate of movement. Although different animals exhibited different absolute values, two intersecting "hyperbolas" were always obtained when time was plotted against temperature. It would be expected therefore that if the logarithm of the rate were plotted against $1/T°$ the points would fall on two intersecting straight lines, indicating the applicability of the so called Arrhenius equation for temperature effects. Separate plots for the 17 animals were so made, and in every case the points could best be represented by two intersecting straight lines. The values of μ (obtained from the slopes of the lines) fell easily into three classes, so that the plots in each class could be combined, it being necessary in some cases to multiply the ordinates by a constant to bring the lines nearer coincidence. These plots, from which a few duplicate points have been omitted for clarity, are reproduced in Figs. 1–3, and show how closely the Arrhenius equation fits the data.

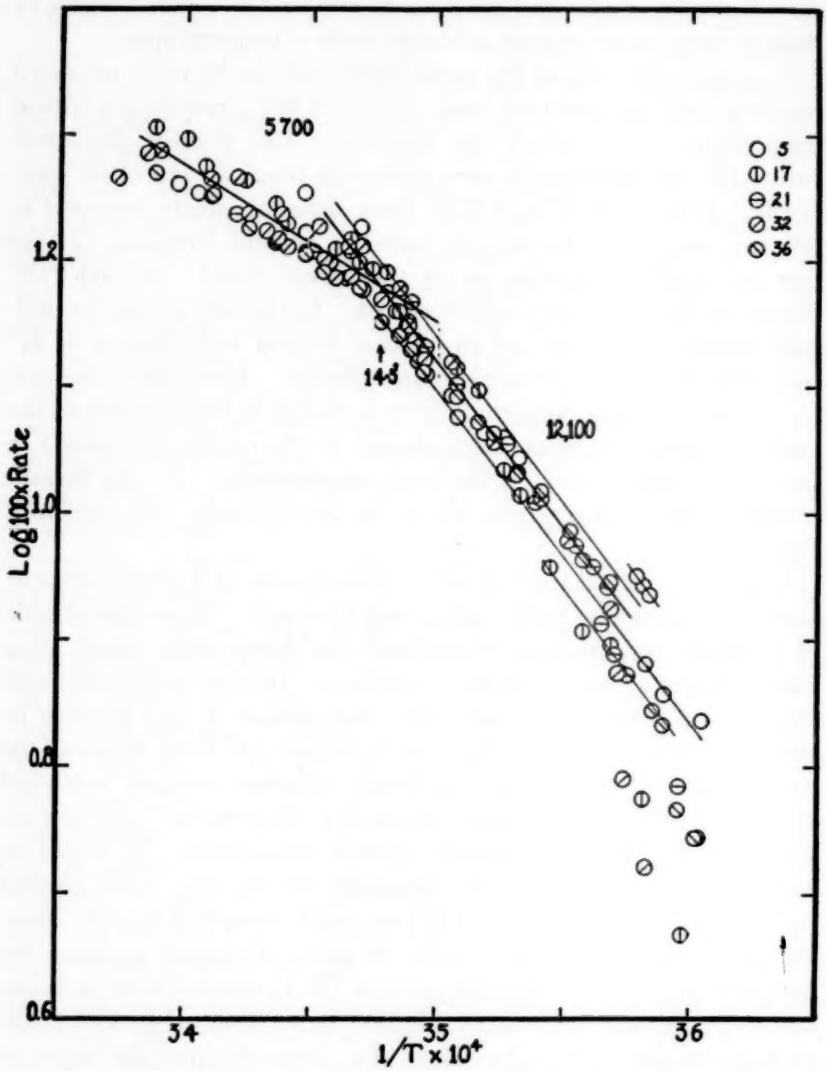

FIG. 1. Data on the rate of the pedal rhythm of *Balanus*, from 5 animals, between 4.4° and 23.4°. Critical temperature is at 14.5° above which μ = 5,700, and below which μ = 12,100. Irregularities appear below 7.0°.

In Fig. 1 are the data from 5 animals, which yielded temperature characteristics of 5,700 from 14.5° to 22.5°, and 12,100 from 7.0° to

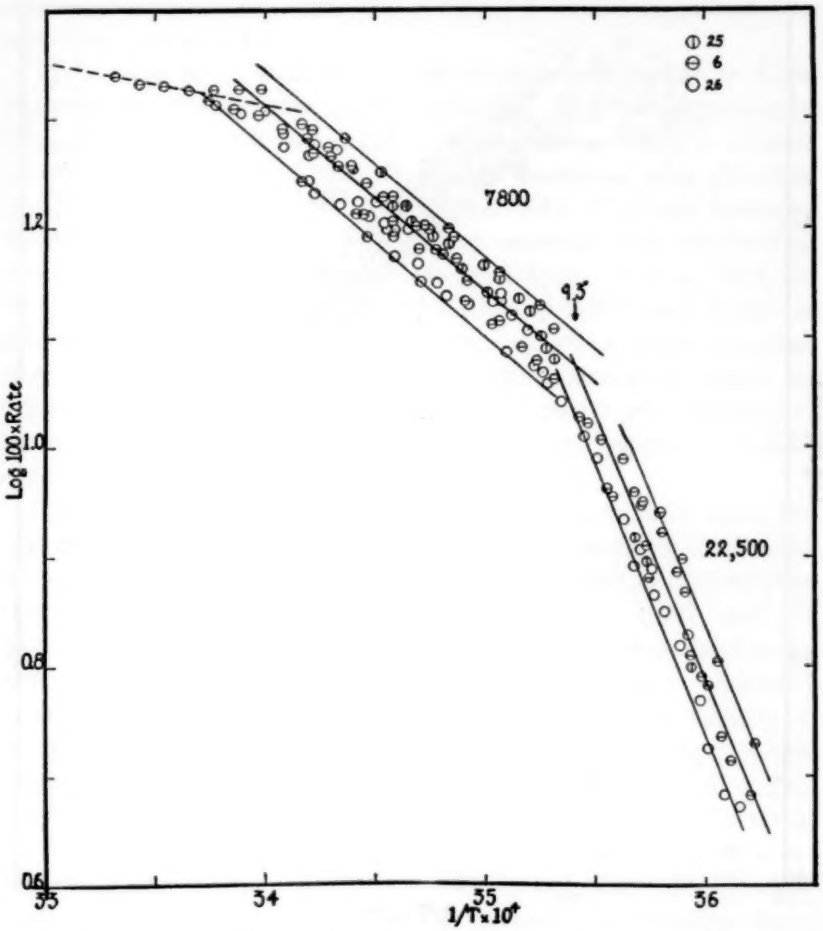

FIG. 2. Data on the rate of the pedal rhythm of *Balanus*, from 3 animals between 2.4° and 27.1°. Critical temperature is at 9.3° above which μ = 7,800 and below which μ = 22,500.

14.5°. Below 7.0° the results were very irregular. In Fig. 2 the values were 7,800 from 9.3° to 21.0°, and 22,500 from 3.1° to 9.3°. Animal 6 in this group continued to show an increasing rate, with a

smaller increment, up to 27.0°, at which closure was sudden. In Fig. 3 the data from 9 animals showed temperature characteristics of

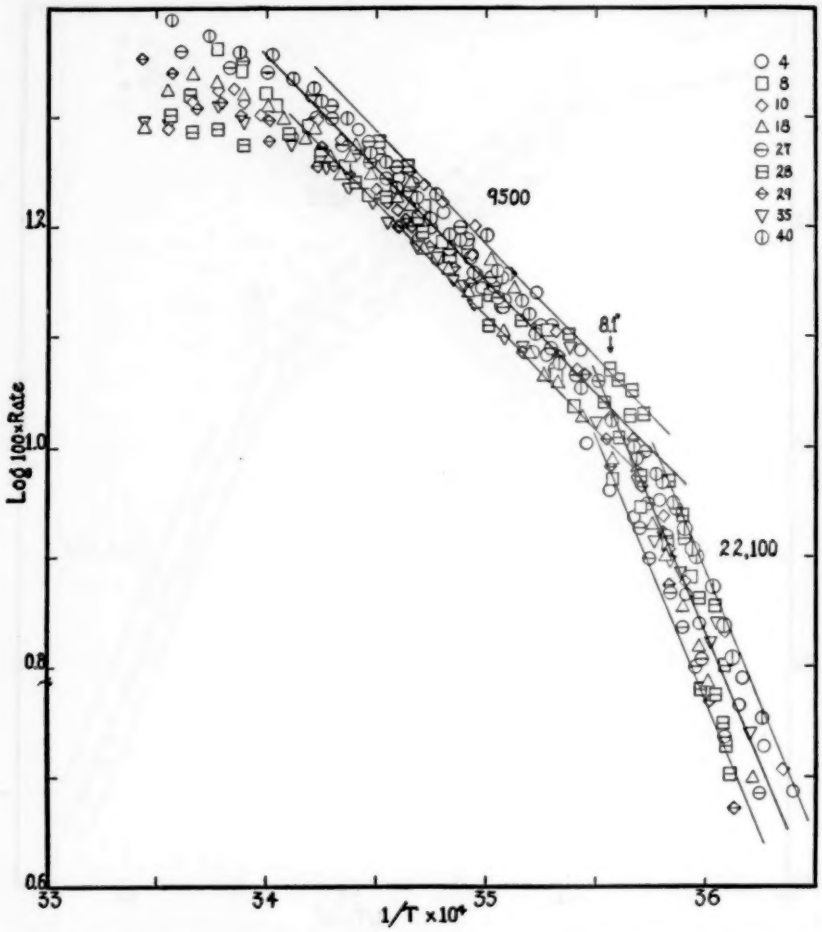

FIG. 3. Data on the rate of the pedal rhythm of *Balanus*, from 9 animals between 1.6° and 25.8°. Critical temperature is at 8.1° above which (to 21.0°) μ = 9,500, and below which μ = 22,100.

9,500 from 8.1° to 21.0° and 22,100 from 2.4° to 8.1°. Above 21.0° the plot showed several rates increasing slowly up to closure. Different temperature characteristics from different animals over the same

temperature range may be due to the fact that different members of a catenary series of reactions which determines the pedal rhythm assume control of the series under differing conditions of age, sex, nutrition, and the like.

The values of μ obtained for the barnacle are similar to those reported for various other animal activities. It is not surprising to find values near 8,000 and 22,000 for a mechanism which is at least partly respiratory, the former frequently associated with processes involving gaseous exchange. The characteristic of 12,100, however, has been associated with non-respiratory neuromuscular rhythms in arthropods (*cf.* Crozier, 1924; Crozier and Stier, 1925, *a*; 1925, *b*). Wolf (1928) found the same combination of 8,000 and 22,500 in normal *Gonionemus* for frequency of pulsation, which is also a respiratory and food-getting function. These three increments might be expected for an activity which is concerned partly with respiration but operated by a neuromuscular mechanism, if the general indications of the results from temperature analyses are sound.

Increments of about 9,000 calories have been previously found for several different organic processes, such as movement of *Oscillatoria* (Crozier and Federighi, 1924; Navez, 1928), heart beat in *Tiedemannia* (Glaser, 1925), and in *Notonecta* (Crozier and Stier, 1927), and for growth of sporangiophores (Castle, 1928). In some of these cases, however, the value appeared only infrequently and was not considered typical for the process. That this 9,500 is not due to a mixture of values near 8,000 and 11,000 is shown by the following series of values calculated from the individual plots: 8,900, 9,030, 9,040, 9,470, 9,560, 9,600, 10,200, 10,370, 10,400, the average of which is 9,620. So far it has been impossible to detect its significance. The records of μ = 5,700 from the barnacle and μ = 4,800 for locomotion of *Planaria* (Cole, 1926) stand by themselves and any attempt to correlate them with known chemical or biological processes must await further investigation; particularly since they occur at such relatively high temperatures. The critical temperatures revealed in the three sets of data are very close to those reported in many other activities. Changes in the control of the rate of a series of reactions frequently occur near 9.5° and 15.0°; near 8.0° such changes are not so common (*cf.* Crozier, 1926).

Fig. 4 is a mass plot of the results from all the animals and illustrates very clearly the inability to interpret temperature effects

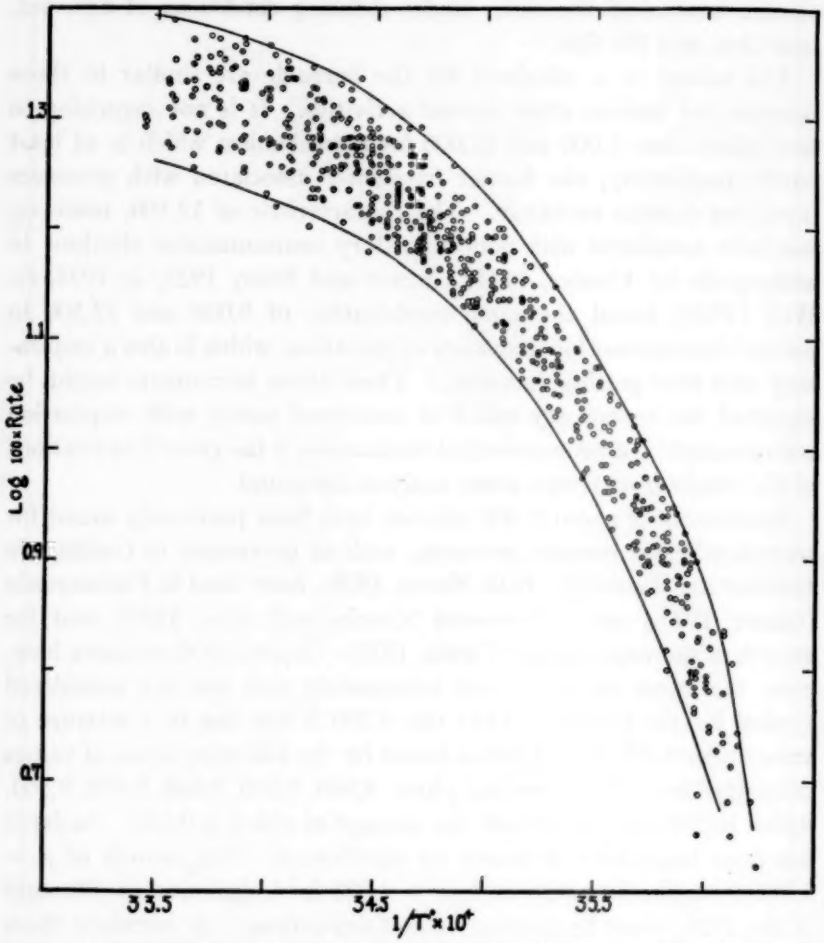

FIG. 4. Data on the rate of the pedal rhythm of *Balanus* from 16 animals between 2.9° and 26.0°, plotted *en masse*. Curvilinear lines indicate different temperature characteristics among the different individuals and the necessity of individual plots.

correctly when the animals show individual differences. The curvilinear shape of the best fitted line is due to the different temperature characteristics over the medium range of temperatures, and to the

varying irregularities above 21.0°C. (Crozier, 1924; Crozier and Stier, 1925, *a*; Glaser, 1924.) If the Arrhenius equation is to be applied to such data individual plots must be made (*cf.* Castle, 1928, page 412; Glaser, 1925).

A second series of experiments was performed to detect any effect of diluting the sea water upon the temperature relations, the pH being kept constant. Enough observations were collected to demonstrate that any dilution greater than 4 per cent caused irregularity of movement; that 3 and 4 per cent dilutions progressively decreased the rates, without altering the temperature characteristics; and that dilutions of 2 per cent or less caused no significant change in rate. The results indicate the extreme sensitivity of this barnacle to fresh water as far as regularity of movement is concerned. It is well known however that much larger amounts of fresh water are tolerated by barnacles without permanent injury, although the pedal rhythm may be temporarily affected.

SUMMARY.

1. The relation of temperature to the pedal rhythm of *Balanus balanoides* L. has been studied under otherwise constant conditions.

2. The frequency of movement increases with temperature, showing three groups of thermal increments and three critical temperatures. Five animals yielded μ = 5,700 above 14.5° C. and 12,100 below; 3 gave μ = 7,800 above 9.3° and 22,500 below; while 9 showed μ = 9,500 above 8.1° and 22,100 below.

3. The upper critical temperatures, above which different effects appeared in different animals were 23.4°, 26.0°, and 27.0°. Above 27.0° none of the valves remained open.

4. Excepting the values 5,700 and 9,500, the increments are similar to those previously found to be associated with respiratory and with neuromuscular activities.

5. Dilution of the sea water with from 3 to 4 per cent fresh water decreases the rate without altering the increments. More than 4 per cent dilution causes irregularity.

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THE RELATION BETWEEN MAXIMUM RATE OF PHOTOSYNTHESIS AND CONCENTRATION OF CHLOROPHYLL.

By ROBERT EMERSON.*

(From the Laboratory of General Physiology, Harvard University, Cambridge.)

(Accepted for publication, January 28, 1929.)

I.

Most investigations of photosynthesis have depended on measurement of the effects of varying certain external conditions on the rate of this process. It is recognized that "internal factors" also have an effect on the rate of photosynthesis. Internal factors are mentioned by both Blackman (1905, p. 281) and Spoehr (1926, p. 161), but owing to the difficulty of controlling them few have tried to investigate the part they may play. Chlorophyll, the most tangible internal factor, has attracted the attention of numerous investigators. Some have tried to study the relation of different concentrations of chlorophyll to the rate of photosynthesis. But no one prior to Willstätter and Stoll accomplished much in this field, chiefly because the nature of the leaf pigments was not understood, and their quantitative determination was impossible.

Willstätter and Stoll, having devised satisfactory methods for the quantitative determination of leaf pigments (1913), undertook to study photosynthesis with leaves containing different concentrations of chlorophyll. They established beyond doubt the fact that during photosynthesis the amount of chlorophyll remains unchanged, even during prolonged exposure to strong light at high temperatures. Pfeffer and Czapek, among others, had suggested that during photosynthesis chlorophyll may be decomposed and reformed constantly. Willstätter and Stoll show that no real decomposition of chlorophyll takes place during photosynthesis. I quote from them (1918, p. 11) the following table:

* National Research Council Fellow.

Chlorophyll in 10 g Blättern vor und nach der Assimilation.
5 proz. CO_2, ungefähr 75000 Lux.

Pflanze	Temperatur und Assimilationszeit	Chlorophyll (mg.)	
		vor der Assimilation	nach der Assimilation
Prunus Laurocerasus	30°, 6 Stunden	12,2	12,4
" "	30°, 22 "	9,4	9,5
Hydrangea opulodes	30°, 6 "	9,2	9,1
Pelargonium zonale	40°, 6 "	12,5	12,8

They show in the same chapter (see p. 32) that not only is the total amount of chlorophyll unaffected by rapid assimilation, but neither is the ratio between chlorophyll *a* and *b*.

Nevertheless, they were unable to establish any relationship between chlorophyll content and capacity for photosynthesis. They used leaves from green and yellow varieties of the same species, yellowing autumn leaves, greening spring leaves, etiolated, and chlorotic leaves. Their results are expressed as amounts of CO_2 reduced per unit amount of chlorophyll. The following figures, quoted from Willstätter and Stoll (1918, p. 43), show some of the widely differing values they obtained:

One molecule of chlorophyll assimilates per hour:

In old autumn leaves of *Ampelopsis quinquefolia* 18 molecules of CO_2.
In young autumn leaves of *Ampelopsis quinquefolia* 164 molecules of CO_2.
In summer leaves of *Sambucus nigra* 135 molecules of CO_2.
In summer leaves of *Sambucus nigra var. aurea* 2463 molecules of CO_2.
In etiolated leaves of *Phaseolus vulgaris* 2736 molecules of CO_2.

The experiments of Willstätter and Stoll were carried out under the usual difficulties involved in working with leaves. The temperature of the cells of an assimilating leaf is never accurately known. The palisade cells of dark green leaves screen the internal and lower cells so that all are not equally illuminated. In yellow leaves, on the other hand, the light is much less weakened in traversing the cells, so they are more equally illuminated.

These may be some of the reasons why the results of Willstätter and Stoll fail to reveal any correlation between amount of chlorophyll and rate of photosynthesis. But in spite of Spoehr's statement

(1926, p. 153) that Willstätter and Stoll have definitely established the disproportionality between chlorophyll content and assimilation, I think we must agree that if chlorophyll really takes part in photosynthesis its concentration should bear some regular relation to the rate of the reaction. The results of Willstätter and Stoll show that among a wide variety of leaves chlorophyll shows great variation in activity. They do not show, however, that such variation exists in leaves chosen to be comparable in all respects other than in chlorophyll content.

The experiments described in this paper were undertaken to see if by using comparable cells, differing so far as possible in chlorophyll content only, some regular relationship between chlorophyll and photosynthesis could be established. Such a relationship, if established, would afford experimental proof that the green pigment chlorophyll is really concerned in photosynthesis.

The advantages of working with a unicellular alga in aqueous suspension have been emphasized by Warburg. For studying photosynthesis at high light intensities suspensions containing very few cells may be used, so that all cells are about equally illuminated. In thin suspensions the temperature of the cells cannot differ significantly from that of the surrounding fluid, which may be kept very constant. On account of its various conveniences, therefore, the green alga *Chlorella vulgaris* was used for this investigation.

II.

Method of Culturing Cells.

Spoehr states (1926, p. 142) that chlorophyll content cannot be artificially varied. However, the chlorophyll content per unit volume of cells of *Chlorella vulgaris* has shown itself to be remarkably sensitive, especially to the composition of the culture medium.

The method of culturing was essentially that described by Warburg (1922, p. 427) in his papers on photosynthesis. As this is already adequately described, I shall add here only such modifications as were used for controlling the chlorophyll concentration per unit volume of cells.

The cultures were grown in 300 cc. Erlenmeyer flasks with tubes

sealed into the tops for passage of a stream of gas. The entrance and exit tubes were plugged with cotton, and the flasks, each containing 225 cc. of medium, were sterilized and inoculated with 3 cc. of cell suspension by means of sterile pipettes. Usually from 10 to 30 c. mm. of cells were inoculated into each flask, each flask in a given set receiving exactly the same amount.

The medium was prepared as Warburg recommends (1922) but only one half the amounts of his stock solutions were used per liter of medium. To dilute the stock solutions, a solution of 1.5 per cent glucose was prepared, and an excess of calcium carbonate added, the suspension well mixed, autoclaved 20 minutes at 13 pounds pressure, mixed again, and allowed to cool and settle slowly. The supernatant glucose solution was decanted and used for diluting the stock solutions. This procedure of autoclaving with calcium carbonate is recommended by Steinberg (1919, p. 358) for removal of traces of heavy metals from nutrient media. It was found that without this treatment chlorophyll concentration could not be varied over so wide a range, probably because of the relatively large amount of iron present in the glucose. Merck's glucose C.P. was used, and best grade Kahlbaum salts; water redistilled from a Pyrex still was used exclusively. The prepared medium was of the following composition:

$MgSO_4$	0.01 molar
KNO_3	0.0125 "
KH_2PO_4	0.0090 "
$CaCO_3$	0.0001 "
Glucose	about 1.5 per cent

A number of experiments were made, a summary of two of which appears in Table I, to determine whether glucose in this concentration depresses photosynthesis. Numerous writers (see Spoehr, 1926, p. 159) have stated that the accumulation of end-products materially cuts down the rate of photosynthesis. It might be expected that a 1.5 per cent glucose solution would show a marked inhibitory effect. No such effect could be demonstrated, the rate of photosynthesis being the same with and without glucose. The respiration of cells in glucose solution was higher, showing that the glucose had penetrated the cells.

The use of glucose in the culture medium of an autotrophic organism

like *Chlorella* is of course unnecessary for growth. But the method used for controlling chlorophyll content was to vary the iron concentration, and glucose was needed to facilitate growth at low concentrations of iron. With only traces of iron present as impurities in the salts and glucose, *Chlorella* soon becomes heterotrophic, loses all its chlorophyll, and continues to grow and live by using glucose. The writer has used this technique of culturing *Chlorella* in glucose solution before (1927). It readily returns to the autotrophic condition and develops chlorophyll if it is returned to a medium with plenty of iron, with or with-

TABLE I.

No. of cells	Suspension fluid	Glucose concentration	Time	O_2 evolved
mm.³		per cent	min.	mm.³
32	Knop*	0	10	43.8
32	Knop	4	10	49.0
10	Carbonate mixture	0	15	27.0
10	Carbonate mixture	1.5	15	27.2

* When Knop solution was used, it was saturated with 5 per cent CO_2 in air.

TABLE II.

Cc. $Fe_2(SO_4)_3$ stock added to culture	Mm.³ cells used to determine Δk	K_{O_2}	Δk	Q_{O_2}	Mm.³ cells extracted	ϕ_1	ϕ_2	ϵ per 10 mm. cells
			per hr.					
0.25	15.5	0.54	+332	+126	36.5	57.5°	34.0°	0.101
0.025	8	0.56	+155	+118	38	57.5°	33.4°	0.099

out glucose. In glucose solutions poor in iron its growth is of course slower than with plenty of iron, but the rate of growth is more nearly the same than if no glucose is used. If one tries to control chlorophyll concentration by growing cultures without glucose and with graded iron concentrations, the cultures poorest in iron grow very slowly, turn yellow and sickly, and show abnormal metabolism, so that they are in no way comparable with cells grown on media richer in iron. For the same reason the leaves of plants grown in water cultures with different amounts of iron are not comparable. Cells deprived of iron, and without sufficient chlorophyll, cannot

remain normal unless they are supplied with carbohydrate from the outside. Glucose was used in these cultures to obtain more nearly equal growth with different amounts of iron. Iron was added from a stock solution containing 2.1 gm. of ferric sulfate per liter. Amounts varying from 0.50 to 0.01 cc. of this stock solution were added to each culture of 225 cc. With this range of added iron, and using glucose, pure cultures of *Chlorella* may be grown at roughly comparable rates, producing cells of about equal size and equal rates of respiration, but differing widely in chlorophyll content.

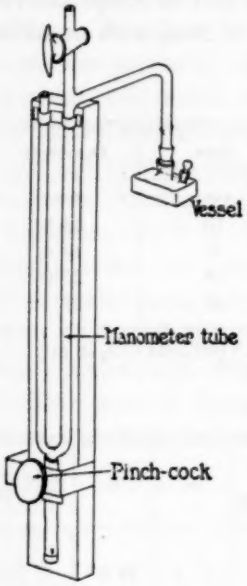

FIG. 1.

The rate of photosynthesis is independent of the iron concentration in the culture, up to the point where iron concentration begins to affect chlorophyll concentration. Up to the age of 3 to 4 days, all cultures produce cells of nearly the same chlorophyll content, no matter what the iron concentration. Table II shows rate of photosynthesis and the chlorophyll content for two cultures, one containing ten times as much iron as the other. Rate of photosynthesis per unit volume of cells and chlorophyll content per unit volume of cells are nearly the same for each culture. After 4 or 5 days, the cultures poorer in iron begin to show lower chlorophyll concentration, and with this the rate of photosynthesis falls off. But external iron concentration alone apparently does not affect rate of photosynthesis.

Cultures were grown in a glass-bottomed water-bath kept at 19–21°C., and illuminated from below with three 40 watt internally frosted concentrated filament lamps about 30 cm. from the bottoms of the culture flasks. A continuous stream of air containing 5 per cent CO_2 was passed through the cultures.

III.

Methods of Measuring Photosynthesis and Chlorophyll Concentration.

Photosynthesis was measured manometrically, by a method well worked out and described by Warburg (1924). Fig. 1 shows the

type of vessel and manometer used. The total volume of each vessel, to the level of the manometric fluid, was about 12 cc. 7 cc. of cell suspension were pipetted into each vessel, so that the total gas space was about 5 cc. Under these conditions, 1 c. mm. of oxygen evolved at 20°C. causes a change of pressure equal to about 2 mm. of Brodie's solution (the fluid used in the manometer). To find the amount of oxygen evolved in photosynthesis, the change in pressure was multi-

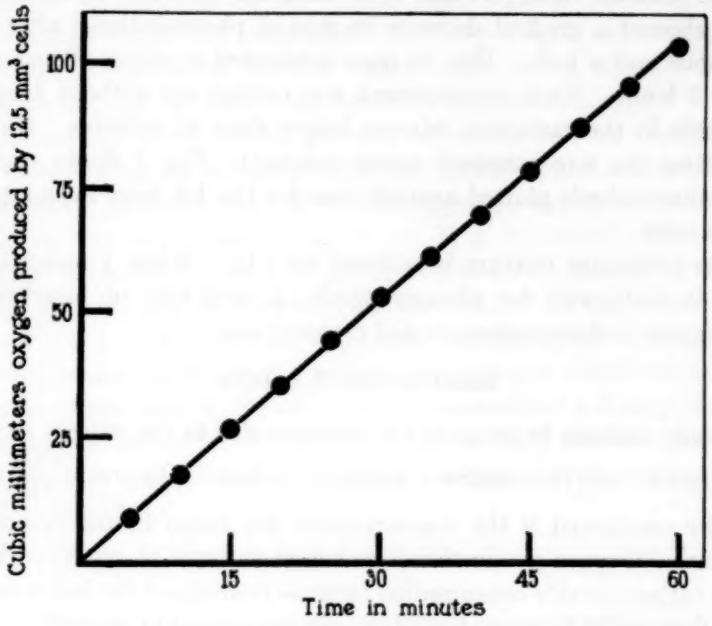

FIG. 2.

plied by a constant, K_{O_2}, characteristic for each vessel for a given temperature and volume of suspension. For the mode of calculation of K_{O_2}, consult Warburg (1924).

The cells were centrifuged out of their culture medium, washed in a mixture of 15 cc. M/10 Na_2CO_3 + 85 cc. M/10 $NaHCO_3$ (Warburg's "Carbonate Mixture No. 9"), and suspended in this mixture for the measurement of photosynthesis. The concentration of CO_2 in this mixture is so high that the rate of photosynthesis is independent of

small changes of CO_2 concentration. The mixture has, according to Warburg (1919, p. 238) an OH ion concentration of $10^{-4.8}$, and contains 91×10^{-6} moles of CO_2 per liter. Variation with temperature is comparatively small, the CO_2 concentration dropping probably to about 71×10^{-6} moles per liter at 5°C. Warburg states that for the organism he used, *Chlorella pyrenoidosa*, this mixture was not injurious for many hours. I found that *Chlorella vulgaris*, although it would continue to respire and to assimilate CO_2 in this mixture for a day, showed a gradual decrease in rate of photosynthesis after the 1st hour and a half. This decrease amounted to about 20 per cent after 5 hours. Each measurement was carried out without keeping the cells in the carbonate mixture longer than 45 minutes. During this time the rate remained nearly constant. Fig. 2 shows amount of photosynthesis plotted against time for the 1st hour for a typical set of cells.

The carbonate mixture is buffered for CO_2. When 1 molecule of CO_2 is withdrawn for photosynthesis, 2 molecules of bicarbonate decompose to form carbonate and carbonic acid:

$$2NaHCO_3 \rightarrow Na_2CO_3 + H_2CO_3$$

The only changes in pressure are therefore due to the oxygen used in respiration and that evolved in photosynthesis. The ratio $\frac{CO_2}{O_2}$ need not be considered if the measurements are taken in the carbonate mixture. Oxygen production is a better measure of photosynthesis than carbon dioxide consumption because readings of the latter might be influenced by fermentation of the glucose present in the cells.

Warburg has calculated the "resistance" of the buffer action (capacity to furnish CO_2 at constant pressure) of this carbonate mixture, and finds that in 10 cc. of carbonate mixture 0.2 cc. of CO_2 may be used up without altering the CO_2 tension more than 10 per cent. The evolved oxygen resulting from the reduction of 0.2 cc. of CO_2 would cause under my conditions a change of pressure of about 400 mm. of Brodie's solution. In no single experiment did the total amount of photosynthesis ever approach this value. Variations in CO_2 concentration were consequently well under 10 per cent.

The most satisfactory source of light for high intensities was found

to be a tungsten ribbon lamp using 6 volts drawn from the 110 volt alternating current lighting circuit by a transformer. The lamp was mounted outside the thermostat, its light made nearly parallel by means of a lens, and thrown on the cell suspension through a plate glass window in the side of the thermostat. In this way intensities of over 100,000 lux were obtained. This arrangement proved to be superior to that involving bulbs immersed in the thermostat, because it made possible the use of intense light without overheating the thermostat water. At times when very intense light was not desired, ordinary concentrated filament lamps were placed directly in the thermostat, their sockets being imbedded in paraffin blocks.

Chlorophyll concentration per unit volume of cells was determined by washing a known volume of cells with distilled water, and extracting with methyl alcohol until all soluble pigment was extracted. The total extract was made up to 25 cc. in a volumetric flask. The extinction coefficient ϵ of a sample of this solution, containing chlorophyll, carotin, and xanthophyll, was determined for the wave-length $\lambda = 670\ \mu\mu$. This point lies completely outside the absorption range of carotin and xanthophyll, and is in the region of maximum absorption by chlorophyll in the red. ϵ was measured on a König-Martens spectrophotometer, the two angles ϕ_1 and ϕ_2 being read at equality of the halves of the field. The glass block used in the absorption vessel was 10 mm. thick. Therefore

$$\epsilon = \log \tan \phi_1 - \log \tan \phi_2$$

If x is the number of cubic millimeters of cells extracted $\frac{\epsilon_{670}}{x} \times 10$ is the extinction coefficient of the chlorophyll of 10 c. mm. of cells made up to 25 cc. in methyl alcohol. This value, ϵ per 10 c. mm. of cells, is proportional to the concentration of chlorophyll alone, because the yellow pigments do not absorb in this part of the spectrum. The values of ϵ given in the following pages are of course relative, but they may be translated into absolute units at any time by measuring ϵ for an alcoholic solution of chlorophyll of known concentration. No attempt was made to determine chlorophyll (*a*) and (*b*) separately. Willstätter has shown that their ratio is relatively constant wherever chlorophyll occurs, and that it remains unchanged during photosynthesis.

IV.

The Relation of Chlorophyll Concentration to Rate of Photosynthesis.

Sets of from 3 to 5 cultures were grown for periods of from 5 to 7 days; to each culture of 225 cc. was added an amount of ferric sulfate varying from 1.05 mg. down to zero. Each culture of a given set was started with the same amount of inoculum. When the cultures had developed enough to show a good range of chlorophyll concentrations, all were removed from the illuminated bath where they were grown, and placed in the dark in the refrigerator, a procedure which did not impair their photosynthetic capacities. The rate of photosynthesis at high light intensity was determined for a sample of

TABLE III.

Culture No.	Cc. Fe stock solution added to culture	Rate of respiration mm.^{3} O_2 used for hr. per 10 mm.^{3} cells	κ_{670}
1	0.0	11.0	0.036
2	0.025	6.7	0.045
3	0.050	8.7	0.063
4	0.100	7.7	0.080
5	0.250	7.8	0.085

cells from each culture, three readings being made with each sample. At the same time another sample of cells from each culture was extracted with methyl alcohol for determining chlorophyll concentration. Usually 5 to 20 c. mm. of cells in 7 cc. of carbonate mixture were used for each measurement of photosynthesis. With such thin cell suspensions, the amount of photosynthesis is directly proportional to the quantity of cells, showing that the light is not appreciably weakened by passing through the cell suspension. Larger quantities of cells were used when the chlorophyll concentrations were very low. However, this was permissible because the blue light, which is much more absorbed by the thick suspensions on account of their carotin and xanthophyll content, was present in the light source in such small amounts relative to the light which was unabsorbed by the yellow pigments, that it was of no importance in the rate of photosynthesis. This was established by measuring photosynthesis with the light beam

passing through an orange glass filter cutting out all the light absorbed by the yellow pigments. Removal of the orange glass caused no change in the rate of photosynthesis.

Details of the measurement of photosynthesis were as follows: A measured amount of cell suspension, containing a known volume

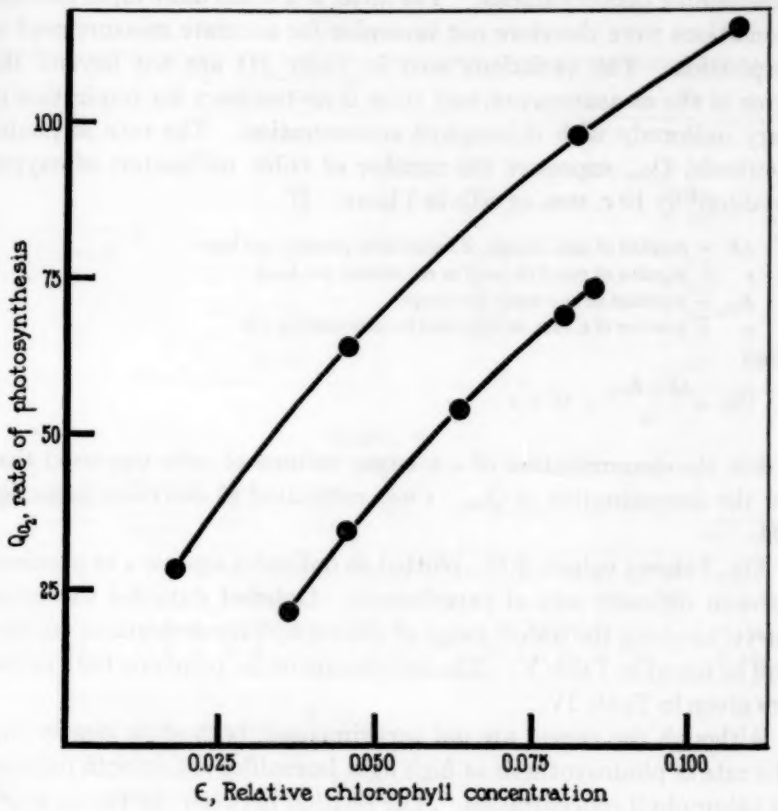

FIG. 3.

of cells, was centrifugally washed with the carbonate mixture, and made up to a definite volume with the carbonate mixture. 7 cc. of this suspension were pipetted into a vessel, the latter was connected with its manometer, and illuminated in the thermostat for a preliminary period of 15 minutes. Readings were taken at intervals of 5

or 10 minutes. It was found that respiration did not vary with chlorophyll content, so a uniform correction for respiration was made. Table III shows rates of respiration for four different concentrations of chlorophyll. It must be remembered that these values for "rate of respiration" were obtained from thin cell suspensions, made up for determining photosynthesis. The latter is a much more rapid process. Conditions were therefore not favorable for accurate measurement of respiration. The variations seen in Table III are not beyond the error of the measurements, and there is no tendency for respiration to vary uniformly with chlorophyll concentration. The rate of photosynthesis, Q_{O_2}, expresses the number of cubic millimeters of oxygen produced by 10 c. mm. of cells in 1 hour. If

Δh = number of mm. change of manometer pressure per hour
r = number of mm.^{3} O_2 used in respiration per hour
K_{O_2} = constant of the vessel for oxygen
n = number of c. mm. of cells used for determining Δh

then

$$Q_{O_2} = \frac{\Delta h \times K_{O_2}}{n} \times 10 + r$$

For the determination of ϵ a larger volume of cells was used than for the determination of Q_{O_2}. ϵ was calculated as described in section III.

Fig. 3 shows values of Q_{O_2} plotted as ordinates against ϵ as abscissæ, for two different sets of experiments. Detailed data for the upper curve, covering the widest range of chlorophyll concentrations studied, will be found in Table V. The coordinates of the points on both curves are given in Table IV.

Although the curves are not superimposed, both show clearly that the rate of photosynthesis at high light intensities is a smooth function of chlorophyll concentration. The relation between the two is nearly linear. It is reasonable to suppose that if all conditions for both sets of cultures had been identical, the curves would be superimposed. However, it would be difficult to maintain such factors as the age, and the amount and character of the inoculum constant from one set of culture vessels to another. Furthermore, it is a matter of minor importance whether the curves of different sets are superimposed. The important point is that in every set studied, the points lie on a smooth curve similar in character to the two depicted in Fig. 2.

It is easy to see how such divergent values as were published by Willstätter and Stoll could be obtained by comparing points on one curve of Fig. 3 with points on the other curve. Take for example the two lowest points. The lowest point on the shorter curve represents about twice the chlorophyll content of the lowest point on the

TABLE IV.

	Cc. $Fe_2(SO_4)_3$ solution added to culture	Q_{O_2}	ϵ_{670}
Curve A	0.000	28.4	0.018
	0.010	64.0	0.045
	0.025	91.0	0.082
	0.250	115.0	0.107
Curve B	0.000	21.6	0.036
	0.025	34.4	0.045
	0.050	54.0	0.063
	0.100	69.0	0.080
	0.250	73.4	0.085

TABLE V.

Culture	Cc. Fe solution added	Mm.³ cells used for rate determination	Mm.³ cells extracted	Δh mm. per 10 min.	K_{O_2}	r	Q_{O_2}	ϕ_1	ϕ_2	ϵ_{670} per 10 mm.³ cells
1	0.00	7	14	+ 4.5	0.53	8	28.4	43.8°	42.1°	0.013
2	0.010	12.5	19.0	+21.5	0.54	8	64.0	46.9°	41.3°	0.045
3	0.025	9.5	19.0	+23.5	0.56	8	91.0	48.7°	38.5°	0.082
4	0.25	6.6	26.4	+19.6	0.60	8	115.0	52.3°	34.1°	0.107

$$\epsilon = \frac{\log \tan \phi_1 - \log \tan \phi_2}{\text{mm.}^3 \text{ cells extracted}} \times 10$$

$$Q_{O_2} = \frac{K_{O_2} \times \Delta h \text{ per hour}}{\text{mm.}^3 \text{ cells}} \times 10 + r$$

longer curve, yet the rate of photosynthesis is slightly lower for the higher chlorophyll content. Equal amounts of chlorophyll do not cause equal rates of photosynthesis when one set of cultures is compared with another. The curves of Fig. 3 present, I believe, the first direct piece of experimental evidence that chlorophyll is the pigment concerned in photosynthesis. Clearly the rate of this process, besides depending on various external factors such as temperature, illumination, and carbon dioxide, is also a function of the amount of chloro-

phyll present per unit volume of cells. But to bring out the regularity of this function, it is essential that material of different chlorophyll content be strictly comparable in other respects.

The quantity ϵ, referred to as *chlorophyll concentration*, does not necessarily indicate concentration in the chemical sense, but only the amount of chlorophyll present per unit volume of cells. Nothing is known about the concentration of the pigment inside the individual cells. Nevertheless, it may be pointed out that the shape of the curves of Fig. 3 is similar to the shape of curves of rate of photosynthesis as a function of the true concentration of external factors (carbon dioxide and light intensity).

It seems essential to consider chlorophyll in relation to these other factors in any attempt to explain the mechanism of photosynthesis. A paper covering experiments on photosynthesis as a function of temperature and of light intensity at different concentrations of chlorophyll follows the present one.

SUMMARY.

Willstätter and Stoll have studied the rate of photosynthesis as a function of chlorophyll content. However, their experiments fail to reveal any regular relationship between these two quantities, probably because, in order to obtain material differing widely in chlorophyll content, they were obliged to use leaves which were not comparable in other respects. The writer describes a method for varying the chlorophyll content per unit volume of cells of *Chlorella vulgaris*, maintaining other factors constant.

Experiments are described which show that the maximum rate of photosynthesis is a smooth function of the chlorophyll content.

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PHOTOSYNTHESIS AS A FUNCTION OF LIGHT INTENSITY AND OF TEMPERATURE WITH DIFFERENT CONCENTRATIONS OF CHLOROPHYLL.

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I.

The writer has shown in a previous paper (Emerson, 1929) that the rate of photosynthesis of *Chlorella vulgaris* is a smooth function of the amount of chlorophyll per unit volume of cells. A relationship between rate of photosynthesis and chlorophyll content being established, it seemed advisable to vary chlorophyll in connection with other factors. As Willstätter (1918) points out, this is the only way of finding out the rôle played by chlorophyll in the photosynthetic process. He suggests that it may function in other ways besides its more obvious part in the absorption of light.

Willstätter and Stoll (1918) made measurements of the rate of photosynthesis of leaves containing different amounts of chlorophyll, at different light intensities and temperatures. They concluded that the yellow leaves were more affected by light intensity, the green leaves more by temperature. The writer has pointed out (1929) that their choice of leaves as material for a study of this kind was unfortunate because of the large number of variables introduced. Their results are probably due to other variables besides chlorophyll content, and do not really indicate properties of the photosynthetic process as a function of chlorophyll content. For this reason it was thought desirable to repeat the experiments of Willstätter and Stoll with material more nearly comparable in all respects except chlorophyll content.

The culture methods for producing *Chlorella* cells differing in chlorophyll content, the method of measuring photosynthesis, and the deter-

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mination of chlorophyll per unit volume of cells, are all described in a preceding paper (Emerson, 1929) and need no discussion here.

II.

The Rate of Photosynthesis as a Function of Light Intensity at Different Concentrations of Chlorophyll.

The rate of photosynthesis as a function of the light intensity has been studied by numerous workers (*e.g.*, Pantanelli, 1903; Blackman and Matthaei, 1905; Blackman and Smith, 1911; Warburg, 1919; Harder, 1921). The curve published by Warburg (1925) may be taken as typical. The rate of photosynthesis increases first rapidly with increasing light intensity, then more slowly. At very high light intensities the rate of the process is practically independent of this factor. Willstätter and Stoll (1918) state that to bring the rate of photosynthesis to this maximum, a higher intensity of light is required for yellow leaves than for green leaves. They plot (p. 149) rates of photosynthesis as ordinates against intensity in lux as abscissæ, for leaves of normal and yellow varieties of the elm. Their figure shows that at an intensity of about 24,000 lux the curve for green leaves has bent over parallel to the abscissa, while the curve for yellow leaves is still rising. Their explanation of this is that the chlorophyll in the upper cells of the greener leaves screens the chlorophyll of the lower cells, preventing the latter from reaching their full capacity. The chlorophyll of the upper cells is assumed to be supplied with more light than it can use, even at lower intensities. This would account for a lower efficiency of greener leaves, but could hardly explain the observation that green leaves reach their maximum rate of photosynthesis at a lower intensity of light than yellow leaves. One would rather expect the reverse to be the case, since in yellow leaves there would be very little screening of the lower cells, and *all* the chlorophyll should receive as much light as it could use at a relatively low intensity. Willstätter and Stoll were unable to get an intensity high enough to cause maximum photosynthesis in the yellow leaves. By bringing their lamp very close to the assimilation chamber they achieved intensities of about 75,000 lux, but at the same time they could not avoid overheating the leaves.

The writer used an arrangement already described (1929) whereby intensities of over 100,000 lux were obtainable without overheating the cell suspension. The rays of a tungsten ribbon lamp running at 6 volts and about 17 amperes were collected by means of a lens, made nearly parallel, and directed to the assimilation vessel with mirrors. The vessel was filled with a thin cell suspension (10 to 20 c.mm. of cells in 7 cc. of carbonate-bicarbonate mixture) so that danger of heating through excessive absorption of light was avoided; the temperature was kept constant to within 0.1°C. With such thin suspensions, the rate of reduction of carbon dioxide is directly proportional to the number of cells used, showing that all cells are practically equally illuminated.

The usual method of varying the intensity of the light by changing the distance of the source from the assimilating cells is unsatisfactory at best, because no source can be regarded as a "point source of light," and the nearer it is placed the greater is the deviation from the inverse square law. The method was inapplicable here because parallel light was used. To vary the intensity, non-selective filters of known transmissivity were used. They were sheets of gelatine containing different amounts of carbon, and cemented between parallel sided pieces of colorless glass. For the visible spectrum these filters are non-selective. A set transmitting 5, 10, 25, 50, and 75 per cent of the incident light was used. By using these singly or combined in pairs, the unscreened light intensity of over 100,000 lux was stepped down to 1/400 of this value in seven steps.

The rate of photosynthesis was measured over three successive 5 minute periods at each light intensity. A measurement of respiration, in the dark, followed these three determinations. It was found that the rate of respiration was independent of the intensity of the immediately preceding illumination, so a uniform correction for respiration was made. To avoid the necessity of making a correction for decrease in rate of photosynthesis with time, a fresh set of cells was used at each intensity of light. During an experiment the stock suspension was kept in the refrigerator. Suspensions may be kept in the refrigerator for 36 hours or longer without having their photosynthetic capacity at 20°C. impaired or altered. An adapting time of 15 minutes was found to be sufficient at all light intensities.

From each culture studied, a sample of cells was extracted with methyl alcohol, and the extract made up to 25 cc. The extinction coefficient ϵ_{670} was determined with a König-Martens spectrophotometer. ϵ represents the relative chlorophyll content per 10 c.mm. of cells.

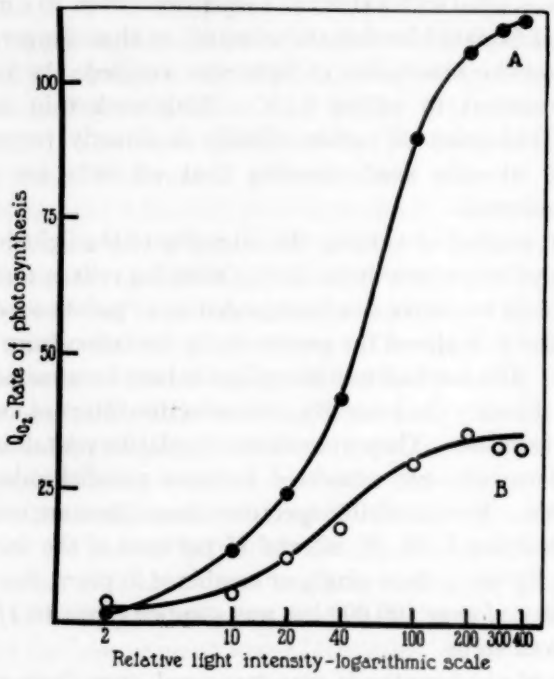

FIG. 1. Curves showing rate of photosynthesis plotted as a function of light intensity, for high and low chlorophyll content per unit volume of cells.

Q_{o_2}, the number of cubic millimeters of oxygen produced per hour by 10 c.mm. of cells, representing the rate of photosynthesis, was plotted against the logarithm of the relative light intensity. The usual method of plotting rate directly against intensity spreads the curve out unnecessarily along the abscissa, and the semilogarithmic plot is more comprehensible.

A large number of experiments were performed, with slight variations in procedure. The results from these individual series led al-

ways to the conclusion that photosynthesis reached its maximum rate at about the same intensity of light, no matter what the chlorophyll content. This is illustrated for two extremes of chlorophyll content in Fig. 1. The value of ϵ for the upper curve is 0.099, for the lower curve 0.026. The coordinates of the points in Fig. 1 are given in Table I, and a summary of the experimental details is given in Table V. The figure shows that both curves bend over toward the abscissa at about the same intensity of light, although they rise to very dif-

TABLE I.

Data for Fig. 1.

	O_{2_3}	Per cent transmission of filters used	Relative light intensity	Log I
Curve A	112	100	400	2.602
$\epsilon = 0.099$	111	75	300	2.477
	106	50	200	2.301
	90	25	100	2.000
	42	10	40	1.602
	24	5	20	1.301
	13.5	5, 50	10	1.000
	2.1	5, 10	2	0.301
Curve B	32.5	100	400	2.602
$\epsilon = 0.026$	33.1	75	300	2.477
	35.5	50	200	2.301
	33.5	25	100	2.000
	17.8	10	40	1.602
	12.5	5	20	1.301
	5.7	5, 50	10	1.000
	4.3	5, 10	2	0.301

ferent heights. It must be remembered that the scale of the abscissa is logarithmic. The flatness of the tops of these curves would be even more evident in a direct plot. It is interesting to note the shapes of these two curves. They are quite dissimilar, and the upper one cannot be produced by multiplying the bottom one by a constant. The curves start at about the same level, showing that at low light intensities the same amount of photosynthesis may be carried on by much or little chlorophyll. The chlorophyll is more efficient when dilute.

This gives us a key to the concentration of chlorophyll *in the plastid.* If we should vary the amount of chlorophyll in the suspension, instead of chlorophyll content per unit volume of cells, we could make two curves using different amounts of cells alike in chlorophyll content. This would give us two exactly similar curves with ordinates the same distance apart from end to end. Nevertheless, the *amount* of chlorophyll for the lower curve might have been the same as for the lower curve of Fig. 1, only it would be differently distributed in the chloroplasts. The difference in shape of the curves in Fig. 1 is attributable to a difference in the state or condition of the chlorophyll *in the plastid.*

These curves bring out another point which seems to have escaped notice heretofore. At very low light intensities (about 500 lux) the rate of photosynthesis is relatively independent of changes in intensity. At intensities of over 1000 lux, the rate of the process varies rapidly with intensity, and again at very high intensities (around 75,000 lux) the rate becomes comparatively independent of the light intensity. The behavior of the process at intensities above 1000 lux is well known, but it seems that measurements below this value are wanting.

The finding of Willstätter and Stoll that the maximum rate of photosynthesis requires a higher light intensity at lower chlorophyll concentrations must have been due to other differences in their material.

III.

The Rate of Photosynthesis as a Function of Temperature at Different Concentrations of Chlorophyll.

The curves shown by Willstätter and Stoll (1918, p. 155) indicate that leaves poor in chlorophyll are much less sensitive to temperature than greener leaves. Their temperature coefficients for green leaves run above 0.2 higher than for yellow leaves. They say that although both types of leaf are equally well equipped with the "protoplasmic factor," on which it is supposed that temperature acts, the leaf poor in chlorophyll cannot absorb light energy fast enough to keep pace with the "protoplasmic factor" at higher temperatures. This explanation is in accordance with Blackman's view of photosynthesis as a process the rate of which is determined by that factor which is most wanting (F. F. Blackman, 1905). He recognized that the rate

of photosynthesis is affected by temperature at high light intensities only. This has been taken to indicate that at least two fundamentally different reactions are involved in the process of photosynthesis. One of them, a photochemical reaction, depends for its pace on the light intensity, and is not perceptibly affected by temperature. The other reaction is purely chemical, and is markedly affected by temperature. The effect of temperature on the rate of photosynthesis is not manifest until light of sufficient intensity is used to make the photochemical reaction exceed the chemical reaction in speed. The chemical reaction is usually designated as the "Blackman reaction," and it is supposed to be enzymatic in nature (*cf.* Warburg, 1925).

Willstätter supposes that in his leaves with low chlorophyll concentrations the photochemical reaction is slower than the Blackman reaction, even at high light intensities. If this explanation is complete and correct, it should be possible to separate the Blackman reaction from the photochemical reaction by changing the chlorophyll content as well as by changing the light intensity. Warburg (1925) gives a condensed summary of the characteristics of the Blackman reaction and the photochemical reaction. The characteristics of the former appear when photosynthesis is studied at high light intensities, of the latter when the process is studied at low light intensities. As pointed out already, the most distinctive characteristic of the Blackman reaction is its sensitivity to changes in temperature, to which the photochemical reaction is relatively insensitive. Another distinguishing feature of the Blackman reaction is that it is easily inhibited by dilute prussic acid, the photochemical reaction being relatively insensitive to the same concentrations of prussic acid.

Extremes of light intensity presumably bring about the characteristics of one reaction or the other by making the photochemical reaction slower or faster than the Blackman reaction. It should be possible to achieve the same effect by using different amounts of chlorophyll, the substance absorbing the light. The results of Willstätter and Stoll outlined above suggest that this is the case. But it seems desirable to compare their results with some obtained from more nearly comparable material.

The published data on the rate of photosynthesis at different temperatures is not adequate for a comparison of this sort. The results

of Blackman and Matthaei and of Willstätter and Stoll are open to the objection that the temperature of the assimilating cells cannot be accurately determined in leaves. Harder (1915), Warburg (1919), and Yabusoe (1924) avoided this difficulty by working with water plants. But Harder's results are inexact because he used light intensities and carbon dioxide concentrations which were too low to cause maximum photosynthesis at the higher temperatures. His curves show that increasing either of these factors caused an increase in rate, whereas in order to study the Blackman reaction, carbon dioxide concentration and light intensity must be so high that the rate of photosynthesis is independent of small changes. Besides, Harder allowed the temperature to vary a degree or more during some readings.

The experiments of Warburg and Yabusoe on *Chlorella* are not open to these technical objections. But their readings are taken so far apart on the temperature scale that it is not possible to determine what sort of function of temperature the Blackman reaction is. Yabusoe, for instance, gives readings taken at 10°, 20°, and 30°, and finds that when the rate of photosynthesis is plotted against degrees Centigrade these three points lie on a straight line. Since the points are so few and widely separated, his conclusion that the Blackman reaction is a linear function of the temperature seems hardly justified.

It is necessary therefore to determine how the Blackman reaction of normally green cells varies with the temperature, in order to have something with which to compare the effect of changes in temperature on the rate of photosynthesis of cells poor in chlorophyll.

The measurements of rate of photosynthesis were carried out in the carbonate-bicarbonate mixture as before. At lower temperatures the dissociation constants for the salts in this mixture are smaller, and the carbon dioxide concentration is lower. However, it was found that at the lowest temperatures used this mixture still gave an adequate concentration of CO_2. The rate of photosynthesis remained independent of small changes in CO_2 tension.

A description of the apparatus for temperature control will be found in a paper by Crozier and Stier (1927, p. 503). Temperature was regulated by a sulfur dioxide compressor, activated by a mercury thermoregulator through two telegraph relays. The temperature was kept constant at any chosen temperature to within $\pm 0.05°$. A fresh

sample of cells was used for each rate determination, the stock cell suspension being kept in the refrigerator. Unless the temperatures chosen were less than 2° apart, respiration was determined at every temperature. For temperatures less than 2° apart, respiration was measured at alternate temperatures, and the intermediate values were interpolated. An adapting time of 15 minutes was found to be sufficient at all temperatures.

Many determinations of rate of photosynthesis were made, at various intervals between 4° and 30°. The most satisfactory method of making the determinations was arrived at after numerous trials,

TABLE II.

Data for Curve A, Fig. 2.

Temperature	K_{O_2}	Mm.³ cells used	Δ H per 10 mm.³ cells per hour corrected for respiration	Q_{O_2}	1/T absolute	Log R
°C.						
4	0.59	19	23.7	13.9	0.003610	1.143
6	0.58	19	39.1	22.7	584	1.356
8	0.56	19	55	30.6	559	1.486
10	0.58	19	75	42.8	534	1.631
11	0.57	13.5	82	46.8	521	1.670
12	0.58	13.5	90	52.3	509	1.719
14	0.62	13.5	104	64.3	484	1.808
16	0.55	13.5	137	75.3	460	1.877
17	0.54	8	145	78.5	448	1.895
26	0.53	8	218	115.5	344	2.062

but a summary of all the data obtained suggests nothing at variance with the conclusions drawn from the data finally secured by the most satisfactory method.

Table II shows the rates of photosynthesis, Q_{O_2}, in cubic millimeters of oxygen per hour per 10 c.mm. of cells, for two typical runs, going from about 4° to about 25°. If these values are plotted directly against temperature centigrade, the result is not a straight line, as Yabusoe supposes, but a sigmoid curve. It is easy to see how his three points, two at the ends and one in the middle, could lie on a straight line. But it is more interesting to plot these data according

to the equation first developed by Arrhenius (1889), and which has since then been found applicable to a large number of reactions and processes, both chemical and biological.[1] If R_1 and R_2 are the rates or frequencies of a process at two temperatures, T_1 and T_2 absolute, then the Arrhenius equation states that

$$\frac{R_2}{R_1} = e^{\frac{\mu}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)}$$

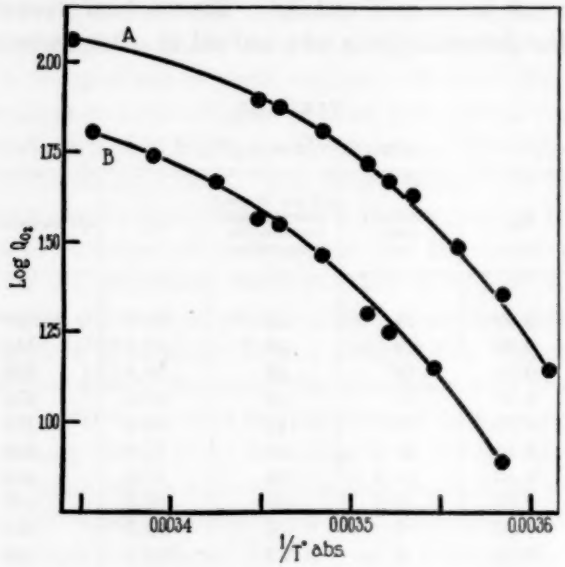

FIG. 2. Curves showing the logarithm of the rate of photosynthesis with two concentrations of chlorophyll plotted against $1/T$ absolute.

If this equation is satisfied, the rate or frequency, when plotted against the reciprocal of the absolute temperature should give a straight line whose slope is μ, a constant found to have characteristic values for certain types of reactions and processes.

Fig. 2 shows the data of Table II plotted according to the Arrhenius

[1] For examples of the Arrhenius equation applied to biological reactions and processes, see papers by Crozier and others in The Journal of General Physiology in recent years. Especially: Castle, 1928; Crozier and Stier, 1927; Wolf, E., 1928; Brown and Crozier, 1927.

equation. The points do not lie on a straight line, but rather on a curve which is concave downwards. The equation is apparently not satisfied. The value of μ decreases gradually from about 31,000 at low temperatures to about 16,000 at high temperatures. Hinshelwood (1926, p. 47) states that if a curve instead of a straight line is obtained from this sort of a plot, it may be concluded that the rate is being determined by the sum of the rates of two or more parallel reactions

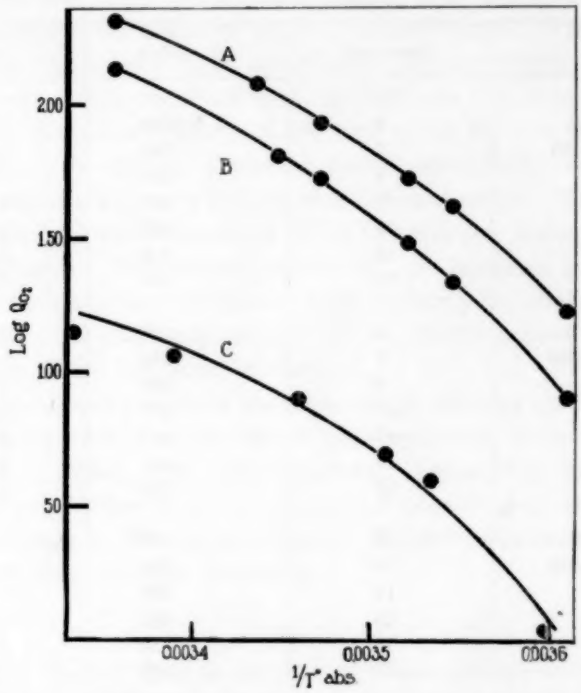

FIG. 3. The logarithm of the rate of photosynthesis plotted against $1/T$ absolute, with three different chlorophyll concentrations.

having different temperature characteristics. He cites as an example the case investigated by Norrish and Rideal (1923) of the combination of sulfur and hydrogen. This takes place in a gas reaction, for which $\mu = 52{,}000$, and a surface reaction on the walls of the container, for which $\mu = 26{,}000$. Hinshelwood's plot of the data of Norrish and Rideal shows a curve which is concave upwards. It is difficult to see

how this could be otherwise, since the higher the temperature the more the reaction with the higher temperature characteristic will predominate. Since the temperature characteristic for photosynthesis *decreases* with rising temperature, it seems unlikely that the case can be explained in the same way as that of Norrish and Rideal.

TABLE III.

Data for Fig. 3.

	Temperature	$1/T$ absolute	Log R
	°C.		
Curve A	4	0.003610	1.230
$\epsilon = 0.101$	9	546	1.622
	9	546	1.592
	11	521	1.728
	15	472	1.932
	18	436	2.093
	25	356	2.318
Curve B	4	610	0.908
$\epsilon = 0.048$	9	546	1.344
	9	546	1.335
	11	521	1.484
	15	442	1.726
	17	448	1.809
	25	356	2.133
Curve C	5	597	0.041
$\epsilon = 0.016$	10	534	0.602
	12	509	0.699
	16	460	0.898
	18	436	1.029
	27	333	1.149

Crozier (1926) in a paper on growth in relation to temperature shows several curves of the logarithm of the rate plotted against $1/T$. They show a uniform tendency to be concave downward, which he suggests is consistent with the autocatalytic nature of the process of growth. If a monomolecular reaction is catalyzed by its end-product, then raising the temperature will affect not only the primary reaction, but also the catalysis of the reaction by its end-product. If the temperature characteristics of these reactions differ numerically, then the

curve of the logarithm of the rate of the entire process plotted against $1/T$ must necessarily be concave downward. It is therefore suggested that the process of photosynthesis may involve an autocatalytic reaction, in which the temperature characteristic for the catalysis differs from the temperature characteristic for the simple reaction.

It is of interest to know whether the shape of the temperature curve will be altered by reducing the chlorophyll content per unit volume of cells. As mentioned above, changing the light intensity does produce a change in the shape of the temperature curve. Fig. 3 shows three curves made with three sets of cells differing widely in chlorophyll content. The coordinates of the points in Fig. 3 are given in Table III. For curve A, $\epsilon = 0.101$, for curve B, $\epsilon = 0.048$, and for curve C, $\epsilon = 0.016$. Curves A and B are strictly comparable. Curve C was made from a culture of a different series. The highest point on curve C was obtained at 27°, a temperature higher than was ordinarily used. Temperatures above 25° are injurious to cells suspended in the carbonate mixture. Their capacity for photosynthesis is impaired after short exposures. This probably accounts for the low position of the top point of curve C.

All three curves have about the same shape, showing that the effect of temperature change on the rate of photosynthesis is the same over this range of chlorophyll concentrations. Decreasing the rate of photosynthesis by lowering the chlorophyll content does *not* produce the same change in this characteristic of the process as decreasing the rate by lowering the light intensity.

III.

The Effect of Prussic Acid on the Rate of Photosynthesis with Different Concentrations of Chlorophyll.

As mentioned in the preceding section, the Blackman reaction is characterized by sensitivity to dilute prussic acid, as well as to changes in temperature. Warburg (1919) found that with decreasing light intensity this sensitivity to prussic acid vanished. In view of the response of photosynthesis to changes in temperature at low concentrations of chlorophyll, it cannot be expected that inhibition of the process by prussic acid will decrease with decreasing chlorophyll content.

Experiments were made by adding free HCN to cells suspended in a solution containing the same salts as the culture medium (KH_2PO_4, KNO_3, $MgSO_4$), or by adding KCN to cells suspended in the carbonate mixture. Both methods produced the same result. The experiments in the carbonate mixture are simpler, and they alone need be described.

When potassium cyanide is added to the carbonate mixture, the concentration of free prussic acid will depend on the amount of cyanide added, on the temperature, and on the hydrogen ion concentration of the solution. Following Warburg, we shall write

C = total cyanide concentration,
H = hydrogen ion concentration,
K = dissociation constant of HCN at the temperature of the experiment,
X = concentration of free HCN

Then,

$$X = \frac{C \times H}{H + K}. \tag{1}$$

Warburg sets $H = 10^{-9.36}$ at 25°C. for the carbonate mixture used in these experiments (Warburg's mixture No. 9). No change in pH could be detected with indicators over a wide range of temperature, so the value at 20° cannot differ appreciably from this figure. Landholt-Börnstein (1912, p. 1138) gives $K = 4.7 \times 10^{-10}$ at 18° and $K = 7.2 \times 10^{-10}$ at 25°. Interpolating, $K = 5.4 \times 10^{-10}$ at 20°. Substituting these values in (1), we find that at 20°

$$X = \frac{C}{2.25}$$

In these experiments a cyanide concentration of 2×10^{-5} moles per liter was used. This gave a prussic acid concentration of about 9×10^{-6} normal.

In each experiment two vessels were used, one without cyanide, the other containing KCN = 2×10^{-5} normal, each vessel containing the same quantity of cells. Thin suspensions were used, and high light intensity was obtained from a 300 watt lamp, immersed in the thermostat directly below the vessels. The light of the tungsten ribbon lamp previously used could not be satisfactorily spread out over two vessels.

Table IV gives the results of one experiment. As the chlorophyll concentration decreases from 0.831 to 0.037, the inhibition by HCN increases from 25 to 40 per cent. Lowering the chlorophyll concentration thus reverses the effect of lowering the light intensity. The sensitivity to prussic acid *increases* instead of decreasing. Photosynthesis continues to show the characteristics of the Blackman reaction when the chlorophyll content per unit volume of cells is lessened, although when the light intensity is cut down the process loses these characteristics. The immediate effect of decreasing either the light intensity or the chlorophyll content is to decrease the rate of photosynthesis. It is supposed that decreasing the light intensity

TABLE IV.

The Effect of Prussic Acid on Photosynthesis at Three Different Concentrations of Chlorophyll.

c	HCN concentration	Mm.^{3} O_2 produced	Inhibition by HCN
			per cent
0.037	0	93.1	40
	9×10^{-6} N	56.2	
0.060	0	122.0	32
	9×10^{-6} N	82.7	
0.083	0	171.5	25
	9×10^{-6} N	129.5	

alters the characteristics of the process of photosynthesis because it cuts down the rate of the photochemical reaction while leaving the Blackman reaction unaffected. The Blackman reaction, then, cannot go faster than the photochemical reaction, and the characteristics of the former cease to appear when the process is studied as a whole. Decreasing the amount of chlorophyll present must also cut down the rate of the photochemical reaction, because chlorophyll is the agent which absorbs the necessary energy for that reaction. But because the characteristics of the Blackman reaction still remain in evidence, it must be supposed that chlorophyll plays a part in that reaction as well as in the photochemical reaction. Unless the concept of photo-

TABLE V.

Detailed Data for Fig. 1.

Absorption data	Filter combination	5–10 per cent	5–50 per cent	5 per cent	10 per cent	25 per cent	50 per cent	75 per cent	No filter
	Relative intensity	2	10	20	40	100	200	300	400
	Log I	0.301	1.000	1.301	1.602	2.000	2.301	2.477	2.602
Amount cells extracted = 52 mm.³ ϕ_1 = 49.3° ϕ_2 = 40.4° ϵ per 10 mm.³ = .026	No. mm.³ cells used	15.5	15.5	15.5	15.5	15.5	15.5	15.5	15.5
	K_{o_2} at 20°C.	0.54	0.55	0.53	0.53	0.56	0.60	0.55	0.54
	ΔH mm. per hr. per 10 mm.³ cells	−10.5	−7.8	4.7	14.7	42.0	42.5	42.0	42.0
	Correction for respiration, mm.³ O_2 per hr.	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0	−10.0
	Q_{o_2}, mm.³ O_2 per hr.	4.3	5.7	12.5	17.8	33.5	35.5	33.1	32.7
Amount cells extracted = 38 mm.³ ϕ_1 = 57.5° ϕ_2 = 33.4° ϵ per 10 mm.³ = .099	No. mm.³ cells used	16	16	16	16	16	8	8	8
	K_{o_2} at 20°C.	0.53	0.54	0.55	0.53	0.55	0.60	0.54	0.55
	ΔH mm. per hr. per 10 mm.³ cells	−15	6.8	26.0	61.0	145	160	187	186
	Correction for respiration, mm.³ O_2 per hr.	−10	−10	−10	−10	−10	−10	−10	−10
	Q_{o_2}, mm.³ O_2 per hr.	2.1	13.7	24.3	42.4	90.0	106	111	112

synthesis as a process involving a Blackman reaction and a photochemical reaction is discarded, it seems to me that this is the only logical conclusion to be drawn from the results presented in this paper.

SUMMARY.

1. Photosynthesis reaches its maximum rate at about the same light intensity over the whole range of chlorophyll concentrations studied.

2. Over this range the process shows the same relationship to temperature. The value of the temperature characteristic decreases gradually as the temperature rises.

3. The rate of photosynthesis is more depressed by prussic acid the lower the chlorophyll concentration.

4. These results are interpreted as indicating that photosynthesis possibly involves an autocatalytic reaction, and that chlorophyll must play some part in the process in addition to its rôle in the absorption of light.

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RESPIRATION AND GEOTROPISM IN VICIA FABA. I.

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The nature of geotropic responses in roots and stems is a problem whereon many people have worked for a long period. Since the first indications of such responses, by J. B. Denis (1673), up to the present time, the number of papers and memoirs dealing with contributions to the theory of this phenomenon has grown to a large figure (about 1,400).[1] And still, we know very little of the intimate character of the processes starting the response and occuring during its execution. We know a little about certain time relations binding together a few variables that we can recognize as significant in the experiments, but we are at present unable to say much about the mechanism of these relations. Even certain points which may be of primary importance seem not to have been touched (*e.g.*, work necessary for curvature, liberation of heat, and the like). Furthermore the advance in physical chemistry in the last 20 years and the new tendencies in general physiology born during the last decade enable one to consider this problem from new viewpoints. It gives also the opportunity to look at things known for a long time with new ideas in mind, and perhaps to gain a clearer picture of the geotropic response.

In the first part of this work we will deal mainly with the respiration of *Vicia faba* seedlings in a state of rest. We will follow also the variations of the geotropic reaction time with change of temperature. From the type of variation so found we may derive indications as to the kinetics of the underlying processes. The second and third parts will deal with variations in the rate of respiration during geotropic response, and with other measurable changes in the responding tissues.

[1] For a careful and complete bibliography of the subject the reader is referred to the work of Miss Christiansen, Bibliographie des Geotropismus, *Mitt. Inst. allg. Bot.*, 1917, and following years.

I.

Conditions of Experimentation.

A.—The device used for measuring the quantities of CO_2 excreted by a single plant or a single root did not depart from the standard methods by more than a few minor modifications. Special care was given to the control of each step in the procedure. A supply of pure air (without even traces of CO_2) was led to a glass respiration chamber immersed in a water thermostat whose temperature was kept constant to within 0.01°C. for any one run. After leaving the respiration chamber the air was bubbled through a Kunz absorption flask containing a concentrated solution of KOH, where all the CO_2 carried over from the plant could be absorbed. Weighings at intervals of 15 or 30 minutes gave the quantities of CO_2 excreted by the plant in the corresponding periods. All the procedure was carried on in a dark room; dim non-actinic red light was the only source of illumination used, and even then for the shortest durations possible.

As anyone will see, the method employed is one very widely used; nevertheless certain details have to be given as to how it was treated. The air which is supplied to the respiration chamber must be pure: it may not contain fumes or traces of H_2S, SO_2, NH_3OH, illuminating gas, etc., in addition to the normal content of CO_2 of the atmosphere. In the apparatus used in these sets of experiments the air was pumped from outside the building, by means of two glass bottles arranged in such a way as to allow a given and constant amount of water to flow from one to the other and thus producing suction. Before entering the metallic piping, absolutely leakproof, which was leading it to the thermostat, the air was freed from dust by passage through 35 cm. of cotton tightly pressed in an absorption tube; furthermore a concentrated solution of mercuric chloride and a 15 per cent solution of H_2SO_4 were freeing the air from the traces of the common gases found. The partially purified air was now passed through a long train composed of (1) three absorption tubes containing soda-lime granules (size No. 4) between cotton plugs, (2) three gas washing bottles with concentrated NaOH solution, (3) one long tube containing a dilute solution of NaOH (4) a tube 45 cm. high containing, to a depth of 35 cm., glass beads and distilled water freed from CO_2 and in which the current of air was introduced under the beads and forced through them, (5) a 12 m. length of copper tubing containing in the two lower spires a small quantity of distilled water. Numbers 2, 3, 4 and 5 were all immersed in the water thermostat where the experiment was performed so as to be in perfect temperature equilibrium. The junctions between the different parts of the apparatus were all glass-to-glass; the rubber tubings, used only where ground-glass joints would have been unpracti-

cal, were heavily coated with a celluloid cement. There was supplied in this way a current of air which, when analysed with the Haldane apparatus, did not show traces of CO_2 or of any of the objectionable gases of a city atmosphere. It was furthermore always saturated with water vapor at the temperature of the thermostat.

The respiration chamber consisted of a 250 cc. gas wash-bottle with fairly wide mouth and long and well ground head piece. The glass tube which normally leads the gas in was bent to one side and used as a support for the plant. This device had the advantage of being very easy manipulated, maintained perfectly sterile, and was possible to place it inside the thermostat in any position, so that geotropic excitation could be applied to the organism studied merely by turning the bottle on its side while all the other conditions remained unchanged. The smallness of the respiration chamber had the advantage of allowing a very easy flushing of all gases liberated in it. The absence in the same chamber of filter paper lining or cotton pads, moistened with water to keep the atmosphere saturated with water vapor (as sometimes used; *cf.* Kuyper, (1910) p. 144; and Fernandes, (1923) p. 121), prevents all solution of CO_2 in this water. The coefficient of partition of CO_2 between water and air is so much more in favor of water that one may expect a certain amount of liberated CO_2 to be held in aqueous solution. That this solution follows the same law as the excretion of CO_2, in relation to temperature, is still to be examined. But in any case there is here a source of serious error which is eliminated by the scheme here adopted. To such cause may be attributed the apparent better constancy shown by the figures of Kuyper (p. 165) in his third experiment, in contrast to his two first experiments performed without addition of water in the respiration chamber. It is also to be noted that the figures corresponding to his third experiment are lower, on the average, than in the other sets.

Furthermore, the use of a Pyrex glass chamber makes one sure that no disturbing reaction in which the copper or aluminium used in some apparatus (*cf.* Kuyper, p. 144), will take place. One may remember the extreme toxicity of copper, especially in soluble salts, and the not less toxic effect of aluminium salts (Szücs, 1913).

A valve was placed between the respiration chamber and the absorption flask to unable us to cut the latter out of the circuit without disconnecting any part of the apparatus. This device was used always at the start of an experiment when the whole circuit had to be flushed and cleaned for ½ hour by a current of pure air, without being obliged to absorb all the possible CO_2 present in the absorption flask.

The pump consisted of two 2 liter flasks with a lower tubulure connected by a rubber tubing. The upper neck opening of flask No. 1 (which was always kept at the same place) was connected through a soda-lime tube to the absorption flask; a stop-cock and a Mohr screw clamp were placed on this connection. The same rubber stopper in the neck of flask No. 1 was fitted also with a small soda-

lime tube normally closed by a stop-cock. When the 2 liters of water had drained from No. 1 to No. 2, it was a simple matter to refill bottle No. 1: the stop-cock on the connection with the absorption flask was closed, the one on No. 1 opened, bottle No. 2 was lifted from the lower position (90 cm. below No. 1) to the upper position (about 75 cm. above No. 1) and the refilling of No. 1 was readily done. To start over again the stop-cocks were turned to their first position. The Mohr clamp was adjusted once for all to obtain a flow of 2 liters of air in 30 minutes; the rate of passage of bubbles through the Kunz absorption flask was in this way adjusted to obtain total absorption of CO_2 in one passage, as was ascertained by tests where a second flask was placed after the normal absorption flask.

The absorption medium in the Kunz flask was a solution of KOH (1 to 1). This solution absorbed naturally also the small amount of water vapor saturating the air current supplied to the respiration chamber; this quantity, which was remarkably constant even over long durations, was determined by a few runs before and after the plant was under observation. It was then a simple matter to correct the readings for the absorption of water.

We have discarded the titrimetric method of estimation of CO_2 absorbed by a solution of $Ba(OH)_2$ with a final titration using phenolphthalein as indicator, on account of a cause of error which seems to have been overlooked. The solubility product of $BaCO_3$ is very small only as long as the solution is strongly alkaline. When the neutralization is proceeding, the equilibrium is not the same any more and a part of the CO_2 may be driven away in a gaseous state out of the solution. Furthermore, as the redissolution of the precipitate of $BaCO_3$ is rather slow, the titration end-point will vary with the speed of addition of the acid. Further still, phenolphthalein has a turning point which is fairly above neutrality.[2]

B.—The plants used in these experiments were young seedlings of *Vicia faba* (Broad Windsor beans), grown in sterile sawdust, in a dark thermostat, at a temperature of 20°C. The seeds had been soaked for 24 hours in running water, disinfected quickly with a 1 per cent solution of formaldehyde or a 3 per cent solution of H_2O_2, rinsed in sterile distilled water and planted immediately. The seedlings were used when the roots reached a length of 4 to 5 cm. (*i.e.*, in the 4th day) and the samples chosen were with perfectly straight roots. The respiration chamber in which the plant was placed was disinfected before and after each run with 94° alcohol and 3 per cent solution of H_2O_2, and rinsed with sterile distilled water. Decay or drying never arose during the runs, and on three occasions the plant was kept for 3 days in the chamber without showing any sign of disease. This indicates that the maintenance of a plant in the respiration chamber for dura-

[2] These points will be reviewed in a note dealing with CO_2 titration.

tions of 24 to 36 hours did not bring into play any uncontrolled factor. In the series of experiments where only parts of the plants were used (root or intact plant attached to a small portion of the cotyledons), all the dissections were made under the red light illumination already mentioned, care being taken also to keep the plant in a normal position during the whole treatment. The cut surfaces were covered immediately with strips or small pieces of cover-glass slightly coated with neutral vaseline. All the manipulations were done as quickly as possible and under aseptic conditions.

As it was thought that these experiments would require a fairly long time before completion, a large stock of seeds was taken so as to have material as homogenous as possible. Several tests performed at different intervals showed that the keeping of the seeds in jars where evaporation was very much prevented did not bring appreciable source of error. The rate of excretion was found to be constant for seeds kept for more than 8 months in the condition described.

C.—In all the experiments care was taken that thorough equilibrium of temperature was attained before beginning the measurements of CO_2 excretion. The plants were allowed to stay for 30 minutes at least in the respiration chamber, normally ventilated with pure air, before the current of air was switched through the absorption flask.

A very extensive series of published and unpublished cases has shown that it is absolutely necessary to have a perfect control of temperature for the thermostat used in such experiments. It must not only be possible to keep any given temperature for any length of time, but also to pass quickly from one temperature level to another and to obtain then the same ease and constancy of control. Furthermore, the thermostat used for biological purposes must be one where the successive temperatures obtainable must be very close one to the other and not only at a few points distributed more or less regularly along the thermometric scale.[3]

The fluctuations of any air thermostat exclude completely this type as too inaccurate. We have used a water thermostat of about 100 liters capacity. A lead coil was supported inside the tank, at the middle of its height, the two ends providing an inlet and an outlet for a current of cold water. This current was regulated so as to cool rather quickly the water of the thermostat which was kept constantly stirred by an electrically driven stirrer. On the other hand, an electric heating unit (with a very large surface and a small mass) was placed on one side of the tank in the whirlpool made by the eddy-currents of the stirrer. The distribution of heat was so obtained in a very efficient way. The heating unit was working

[3] About thermostats *cf.* Crozier and Stier (1926–27).

on A.C. at 110 volts and was thrown in or out of action by a high resistance relay placed in a circuit with two to four dry cells and a large mercury thermoregulator. The volume of the mercury and the diameter of the capillary of the thermoregulator were chosen to give easily a regulation within ± 0.01°C. The temperature was read after each observation made, *i.e.* every 15 or 30 minutes. This arrangement provided a thermostat which was run without any trouble for days and even weeks.

D.—As it is impossible to have two lots of seedlings having all characteristics identical (length of root, weights of different parts, *etc.*), one may wonder to what common base the amounts of CO_2 excreted must be reduced. Will it be necessary to use the *individual* as unit, or *unit weight* (fresh or dry), or *unit surface?* The first may be discarded as being too irrelevant; the latter also on account of the diffi-

TABLE I.

The Relative Proportions of Fresh and Dry Weight of Seedlings of Vicia faba with Their Ranges of Absolute Variation.

		range
Mean fresh weight	5.6450 ± 0.173 gm.	5.012 to 6.460
Mean dry weight	1.8557 ± 0.057 gm.	1.4419 to 2.1714
Mean ratio $\frac{\text{Dry weight}}{\text{Green weight}}$	32.2 ± 1.0 per cent	

culty of ascertaining with any precision the surfaces involved. The only basis that remains is then the weight. But shall we use green weight or dry weight? In fact, as will be seen from the table given (see Table I), the variations of green and dry weights are rather small for the samples used, and as the ratio of dry weight to green weight is fluctuating in still much narrower limits, we will refer all CO_2 excreted to the fresh weights of the seedlings.

It must also be noted that to a very good approximation there is for *Vicia faba* a direct relationship between the CO_2 excreted and the weight of the seedling. It seems to us therefore perfectly proper to reduce our data to a rate of excretion per standard weight of plant and per unit interval of time.

Finally, in the cases where total seedlings or parts of seedlings were to be compared, we have been able to reduce all the quantities found

to the same standard, taking into account that if at any given temperature the total CO_2 excreted by a complete seedling is 100 per cent, the cotyledons give 67 per cent and the plantule gives 33 per cent. Of 33 per cent the stem excretes 18 per cent and the root 15 per cent.

The constancy of these figures is remarkable as they have been found on a number of occasions. In fact this constancy allowed us, in the great majority of cases, to use intact seedlings, which were more easy to handle and which also fitted better our principle of "no interference" with the normal activity of the organism than would the dissecting or removing of parts of it.

II.

The Resting Metabolism.

A review of the literature concerning the relation of respiration of seedlings to temperature is rather simplified by the small number of papers published on this matter. This situation is in fact even remarkable when one takes into account the importance of the processes involved. It must be said, also, especially for most of the older work, that the lack of precision in the experimental conditions (control of temperature, scarcity of the points chosen on the thermometric scale) obliges one, if not to discard practically all these data, at least to consider them for the most part as simply indications.

The first work where a fair constancy of temperature and temperature control are found, are in the papers by Rischawi (1877), Kreusler (1887–88) and Clausen (1890).[4] But it must be said that these authors were more interested in the determination of the "optimum" temperature for respiration than in the exact relation of respiration to temperature. So we find that these papers pay special attention to the higher temperatures and, as was pointed out by Kuyper, the differences existing between these three sets of results can be ascribed to several causes of which one is the treatment of the organisms at high temperature. Clausen especially seems not to have paid enough at-

[4] Nevertheless in Kreusler's work he notes that differences in temperature between two parts of his respiration chamber were 1° to 2°, and in unfavorable cases even 3° to 4°; he also says "so hat das gewiss nicht viel zu bedeuten" (*Landw. Jahrb.*, 1890 xix, 651).

tention to the effect of maintaining plants for a duration of 2 to 3½ hours at temperatures of 35°C. and even higher.

The most elaborate and careful work yet published is that of Kuyper (1910). After having pointed out several mistakes of his predecessors in this line of investigation, he proceeded to show that one of the chief causes of error was precisely that of keeping the plants for long duration at high temperature. The prehandling of the plant determines to a certain extent the results obtained, and in the interpretation of the data a time factor must be taken into consideration. But, as with his forerunners, he also is mostly interested in high temperatures; he attempted to test the applicability of Blackman's theory of limiting factors to normal respiration (cf. p. 139). However, even if his interest lies in the upper part of the thermometric scale, the range 0° to 25°C. is considered with care.

The results of his work (done mostly on *Pisum sativum*, *Lupinus luteus* and *Triticum vulgare*) showed that the respiration rates studied for periods of 6 hours show a very great constancy up to 10°C.; for temperatures up to 20°C. one finds first an increase followed by a decrease in the rate of excretion; for still higher temperatures up to 40°C. the same thing holds true but much more exaggeratedly. The drop in rate seems to follow a logarithmic curve. The critical temperature where one type of respiration (constant) passes to the other type (decreasing) seems to depend on the nature of the reserve substances; being higher for amylaceous reserves, lower for protein reserves. Van't Hoff's rule is valid for *Pisum* and *Triticum* in the range 0° to 20°C., for *Lupinus* up to 25°C. Q_{10} has a value between 2 and 3.

Change in temperature does not "activate" the respiration. The work of Kuyper shows very clearly the existence of critical temperatures above which the phenomenon studied seems to take a new course. This is a point of special interest to us, to which we will have the occasion to come back later. As to the question of applicability of Blackman's theory, as well as about the significance of the Q_{10} obtained, one may differ in opinion. As a whole, this work is the foremost and the data can be accepted as the most precise yet published.

In the experiments here described, all performed with single, entire seedlings, we chose the following temperatures on account of the fact that they cover the range of the thermometric scale where normal geotropic responses could be gotten. These measurements of CO_2 ex-

TABLE II.

CO_2 Excretion by Sets of Five Seedlings at Each Temperature, with the Reductions to 100 Gm. Fresh Weight Per Hour, and the Variability.

Temperature	Weight	CO_2 per seed per hour	Mean	CO_2 per 100 gm. per hour	Mean	Deviation from mean	Mean deviation	Mean deviation as percentage of mean
°C.	gm.	mg.		mg.				per cent
7.5	5.10	1.15	1.29	22.55	23.11	0.56	0.426	1.84
	5.31	1.20		22.60		0.51		
	5.51	1.27		23.14		0.03		
	5.88	1.40		23.81		0.70		
	6.10	1.43		23.44		0.33		
10	5.20	1.57	1.72	30.29	30.154	0.13	0.085	0.28
	5.54	1.67		30.23		0.07		
	5.65	1.70		30.09		0.06		
	5.97	1.80		30.15		0.006		
	6.23	1.87		30.01		0.14		
12	5.17	1.92	2.15	37.14	37.24	0.10	0.082	0.22
	5.36	2.00		37.31		0.07		
	5.70	2.13		37.37		0.13		
	6.18	2.30		37.22		0.02		
	6.46	2.40		37.15		0.09		
15	5.01	2.48	2.73	49.50	49.24	0.26	0.136	0.27
	5.29	2.60		49.15		0.09		
	5.80	2.85		49.14		0.10		
	5.98	2.95		49.33		0.09		
	6.17	3.03		49.10		0.14		
17.5	5.11	3.27	3.57	63.99	63.75	0.24	0.198	0.31
	5.45	3.45		63.30		0.45		
	5.65	3.60		63.72		0.03		
	5.78	3.70		64.01		0.26		
	6.04	3.85		63.74		0.01		
20	5.27	4.35	4.72	82.54	83.10	0.56	0.38	0.46
	5.60	4.65		83.03		0.07		
	5.69	4.75		83.48		0.38		
	5.70	4.77		83.68		0.58		
	6.16	5.10		82.79		0.31		
22.5	5.23	5.15	5.56	98.47	98.77	0.30	0.782	0.79
	5.40	5.40		100.00		1.23		
	5.50	5.45		99.10		0.33		
	5.92	5.75		97.13		1.64		
	6.08	6.03		99.18		0.41		

TABLE II—*Concluded.*

Temperature	Weight	CO_2 per seed per hour	Mean	CO_2 per 100 gm. per hour	Mean	Deviation from mean	Mean deviation	Mean deviation as percentage of mean
°C.	gm.	mg.		mg.				per cent
25	5.18	5.80	6.36	111.96	112.86	0.90	0.64	0.57
	5.32	6.05		113.72		0.86		
	5.70	6.40		112.28		0.58		
	5.88	6.68		113.60		0.74		
	6.12	6.90		112.74		0.12		

cretion were indeed collected in order to study the kinetics of the geotropic curvature. The temperatures were: 7.5°, 10.0°, 12.0°, 15.0°, 17.5°, 20.0°, 22.5°, 25.0°. Higher temperatures were disregarded for the present, for reasons which will be explained later. The normal duration of a single experiment ranged from 6 to 12 hours, during which estimations of the CO_2 excreted were made every 15 or 30 minutes.

For each plant used, the green weight was determined immediately after the run, care being taken also to determine separately the green weight of the cotyledons, stem, and root. These different parts of the seedlings were then dried for 4 to 6 hours at 105°, and after cooling the dry weight was obtained also. This procedure gave us the following results (see Table I).

The distribution of the variations of weight is quite symmetrical. For this reason, when the CO_2 output was obtained for a known weight of plant, the excretion value was reduced to a standard weight of plant of 5.65 gm.

Contrary to what previous observers have claimed about the non-direct relationship between CO_2 output and the weight of the plant excreting it, we have found a very good agreement between the two quantities, as will be seen from Table II.

Another point which seems of interest is that, contrary to what Kuyper (p. 166 and ff.) and Fernandes (1923, p. 170) describe, we could not find that the rate of excretion was changing with time. Outside of the normal, small variations in the readings (which never reached three times the small experimental error), the rate of excretion

was found to be constant, for the periods considered. It must be said that in Kuyper's work the means of appreciation of constancy in the rate of excretion are rather vague. He considers, for instance, that for the temperatures 0°, 5° and 10° the respiration is constant; on the contrary, for the temperatures 15° and 20° the constancy is not the rule. His Table I (p. 166) is intended to illustrate the foregoing. Let us consider it more closely (see Table III).

From the figures given in Table III he concludes that—as the variations lay well in the limits of 1 mg.—the rates at 0°, 5° and 10° are

TABLE III.

Kuyper's Data for Excretion of CO_2 in Successive Hours. The Two Last Columns Are Added to Show the Mean Deviations, and the Mean Deviation as a Percentage of the Mean.

Temperature	1st hr.	2nd hr.	3rd hr.	4th hr.	5th hr.	6th hr.	Mean	Mean deviation	Mean deviation as percentage of mean
°C.									*per cent*
0	4.0	4.0	3.6	4.0	3.8		3.9	0.16	4.10
5	6.7	6.0	5.6	5.7	5.8		6.1	0.38	6.20
10	12.0	11.2	11.1	11.4	11.4	11.3	11.4	0.20	1.75
15	18.6	19.0	19.4	19.4	20.0	20.2	19.4	0.36	1.86
20	28.6	30.3	30.3	30.6	30.4		30.0	0.60	2.00
25	43.3	42.4	42.2	49.7	40.7	40.9	42.0	0.76	1.81
30	51.7	50.9	52.2	53.6	53.5	53.5	52.6	0.96	1.70

constant. But we may object that here, the absolute variation is not a fair indication of variability. The mean deviation as a percentage of the mean would be the index to rely on. The two last columns of Table III (added by us) give a clear indication of a good constancy over the whole range here considered, except at 5°. And even for this last temperature, this does not point necessarily to a lack of *precision* in the measurements. We must give to all the figures the same weight; in such case this deviation may become indicative of some other factor coming into play. This is precisely the type of variation that we get when the temperatures considered are "critical temperatures" for the process involved (*cf.* Crozier, 1924, *a;* Crozier and Federighi, 1924–25). We may add that the second maximum

in these data, reached at 20°, is obtained precisely in a region where a second critical point for this phenomenon is known to be located. The relative smallness of this second maximum can be attributed probably to the fact that we have no data for some intermediate temperatures between 20° and 25°; the critical temperature is in fact, around 21° (*cf.* later), and the value 2.0 per cent may be below the actual

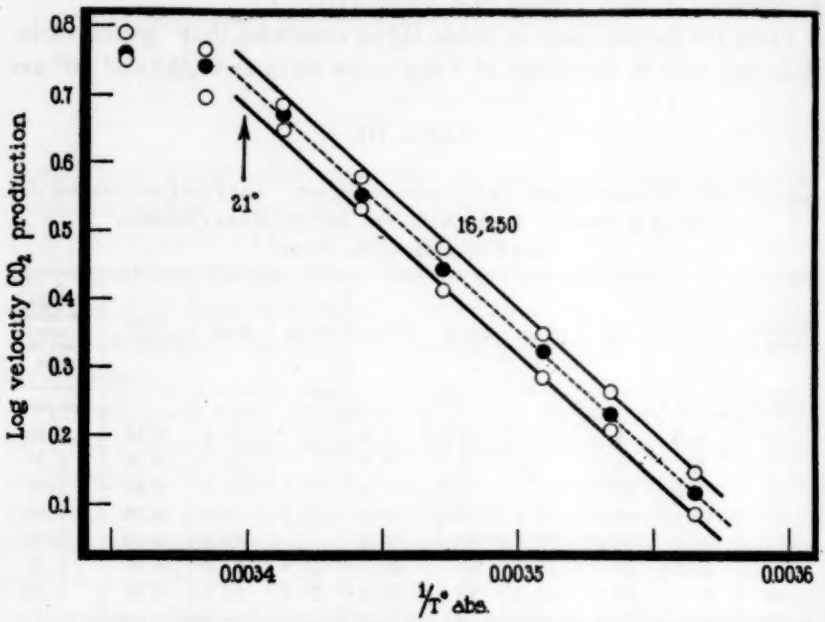

FIG. 1. CO_2 production by seedlings of *Vicia faba*. The broken line corresponds to the line best fitting the data in Fig. 3, the two heavy lines describe the band of variations of the data from which the averages were made, the light circles represent for each temperature the extreme variations. It should be noted also how much the latitude of variation is increased around the upper critical temperature.

maximum of deviation. As has been shown on several occasions by Crozier (1924, *a*), and Glaser (1925–26), the latitude of variation of observations is often much larger at critical temperatures than at any other places in the temperature range (Fig. 1). In this respect, the observations of Kuyper show a remarkable parallelism with ours, if we consider a measure of variability in the rate of excretion to be given by the mean deviations as a percentage of the mean, since in

both cases we find the variability to be maximum at the same temperatures (Fig. 2). As the two methods of measurement and the material used were totally different, the fact that we get such a parallelism points once more to the importance of these critical temperatures. It shows also the absolute necessity, when a biological process is investigated from the viewpoint of its variation with temperature, to have a large number of observations scattered over the whole range

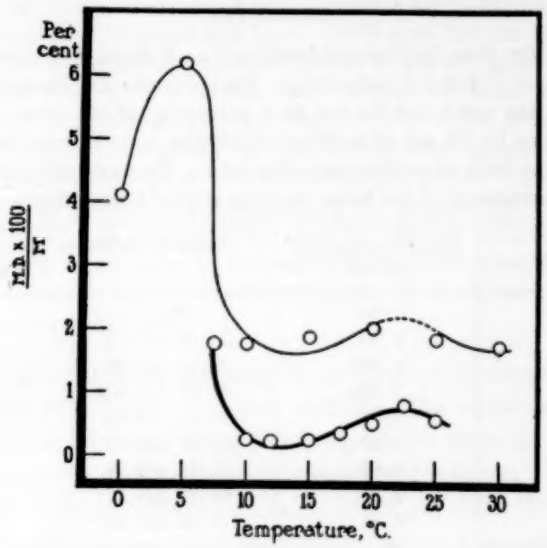

FIG. 2. The relation of the mean deviation as a percentage of the mean, in series of determinations of CO_2 excretion by seeds of *Pisum* (Kuyper: CO_2 mg. per 100 gm. per hour) and of *Vicia faba* (CO_2 mg. per 100 gm. per hour), to temperature, in Kuyper's data (light line) and personal data (heavy line). The absolute values of these deviations are shown also.

studied (and not grouped at 5° intervals, as has so often been the case previously). The summary of most of the data so obtained will be found in Table IV,[5] where all the weights of CO_2 excreted per hour have been reduced in terms of the standard seedling of 5.65 gm.

[5] The slight discrepancies found between Table IV and the figures given in Table II are due to the fact that in the latter case five samples, evenly distributed over the range of seedling weight, were taken. Table IV on the contrary takes into account all the experiments performed.

The measurements so obtained can be utilized in several ways. We may apply to them the method A. Kanitz (1915) used in his book "Temperatur und Lebensvorgänge;" that is to say, we may determine, as has been done for a very large variety of chemical processes, the temperature coefficient Q_{10} for a rise in temperature of 10°C. A good many protoplasmic activities have it in common with a large number of chemical reactions that their velocities are doubled or tripled for a rise in temperature of 10°, at ordinary temperatures. But it may be objected that the Q_{10} is a very inefficient way of characterisation of processes, as it is dependent

TABLE IV.

CO_2 Excretion Per Hour by Standard Seedling (i.e., Reduced to Weight 5.65 Gm.) of Vicia faba, at Different Temperatures. These Figures Are Plotted in Fig. 3.

The P.E. of the mean and the P.E. as a percentage of the mean are also given for CO_2 excretion by 100 gm. of seedlings at different temperatures; the respiration computed on this basis gives the same value for μ. The variability (P.E./M × 100) is a constant percentage of the mean, between critical temperatures.

Temperature	CO_2 per standard seed per hour	Mean CO_2 per 100 gm. seeds per hour	P.E./M × 100
°C.	*mg.*	*mg.*	*per cent*
7.5	1.30	23.20 ±0.12	0.53
10.0	1.705	30.14 ±0.09	0.32
12.0	2.104	37.30 ±0.105	0.28
15.0	2.78	49.32 ±0.15	0.29
17.5	3.60	63.67 ±0.15	0.24
20.0	4.69	82.98 ±0.21	0.26
22.5	5.56	98.82 ±0.37	0.37
25.0	6.38	112.64 ±0.20	0.18

on the range of temperature chosen (*cf.*, for instance, Crozier, 1924–25 *b*, 1925–26 *e*). Kuyper had found for his data the following values of Q_{10}: $\frac{A_{10°}}{A_{0°}} = 2.4$, $\frac{A_{15°}}{A_{5°}} = 3.1$, $\frac{A_{20°}}{A_{10°}} = 2.8$ for one set of experiments (average about 2.8); $\frac{A_{23°}}{A_{13°}} = 2.2$, $\frac{A_{25°}}{A_{15°}} = 2.0$ for a second set of experiments (average about 2.1). For our own data we find $\frac{A_{17.5°}}{A_{7.5°}} = 2.78$, $\frac{A_{20°}}{A_{10°}} = 2.75$, $\frac{A_{22.5°}}{A_{12°}} = 2.6$, $\frac{A_{25°}}{A_{15°}} = 2.29$. The value of Q_{10} is variable and dependent on the interval of temperature considered. Beside the lack of constancy that the Q_{10} shows, this coefficient has another disadvantage: neither from its variations nor from its absolute values may we conclude anything relative to the type of process we study.

We may also handle these data in a graph showing the relation of CO_2 excretion

(ordinates) *vs.* temperature (abscissa), and the curve so obtained can be described by an empirical equation, with two or more constants. But one may question also if such an equation, which can always be written, will necessarily mean something; if the constants with the values there ascribed will represent some definite steps in the process or some characteristics of the system. In fact, in most of the equations belonging to this type, the constants are merely numerical factors having no real significance whatsoever. Such a case is found for instance in the formula given by Bělehrádek (1926): $y = \frac{a}{x^b}$ or $\log y = \log a - b \log x$, where y is the time, x the temperature *centigrade* and a and b are constants. This is the well known formula of Harcourt and Esson (1895) save that for no conceivable reason the absolute temperature has been replaced by the centigrade temperature. Such an equation enables one to fit with some exactness any series of observations where no abrupt change occurs, provided they do not go over a too great range of temperature; it furthermore allows the author in this case to assign to the constants such vague attributions as "viscosity of the protoplasm." For a review of reasons for the inapplicability of such formulæ the reader is referred to Crozier and Stier (1926–27). In résumé, such equations describe a curve but do not depict the process schematised by the curve or the mechanism underlying it. What we are interested in is a description which will throw some light on the kinetics of the process involved.

Numbers of vital activities may be considered as coupled systems of irreversible reactions. To such systems, the equation described by Arrhenius in 1889 may be applied, especially when we know that, although originally given as a purely empirical relation, it was found to submit to rational interpretation (*cf.* Tolman, 1927; Hinshelwood, 1927). This equation assumes that the velocity of an irreversible reaction is directly proportional to the exponential of $\frac{-\mu}{R\,T}$, where R is the gas constant, T the absolute temperature and μ the "energy of activation" per gram molecule of the "activated" substance. This expression, written habitually in the form

$$v_2 = v_1\, e^{\frac{-\mu}{2}\left(\frac{1}{T_1}-\frac{1}{T_2}\right)}$$

with v_1 and v_2 the velocity constants (or proportional quantities) at the absolute temperatures T_1 and T_2, gives μ directly, as from an adequate choice of coordinates and large scale units the value of μ is obtained by a single reading of the [20 inch] slide-rule (*cf.* Crozier, 1925–26, *e*).

This index is independent of the interval of temperature chosen, as well as of the absolute values of the velocities of the process studied. Despite what has been said by some writers, it is not affected by the aging of the organism, as a wide series of experiments in this laboratory has shown. If we consider μ as the activation energy of a catalyst, we may admit that processes activated in the same way will give the same μ; and in the cases where the same catalyst occurs in different reactions

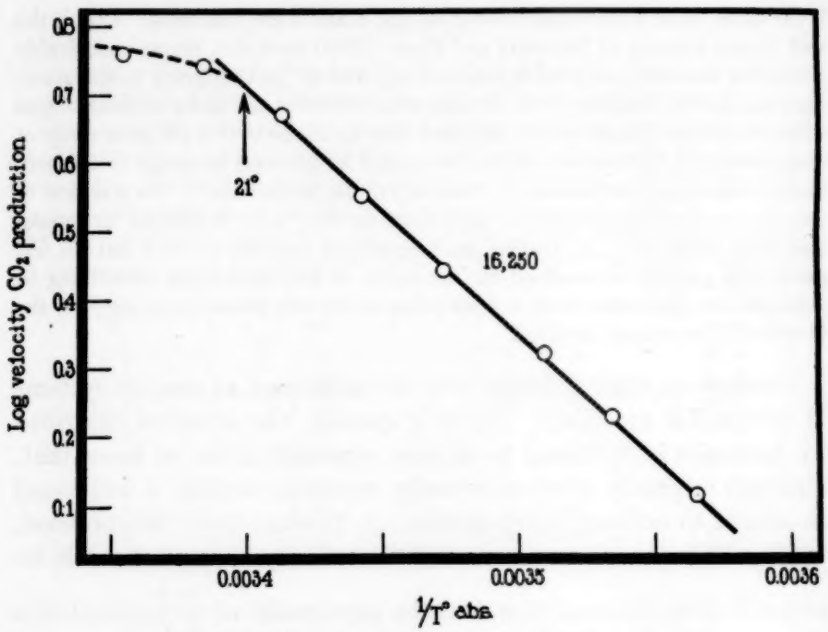

FIG. 3. CO_2 production by seedlings of *Vicia faba*.

we may expect to find the same value for μ. We may thus have an instrument which may give us indications as to the type of reaction controlling a series of coupled reactions. This method as used by Crozier (1924) has been developed with his collaborators in a series of studies which have plainly confirmed this viewpoint. It has furthermore the advantage of being easily applied when the observations have been made with due care; then it constitutes a real criterion of the precision in experimentation and of constancy in the operating conditions.

The data from our observations are rather striking in their good agreement with the form of the Arrhenius equation; the departure from the best fitting line is at any point at most of the order of the probable error. Moreover the value obtained (Fig. 3), $\mu = 16{,}250$, is one more in the list of oxidative respiratory processes yielding a critical increment of about 16,200. Compare this for instance with the values found in the published data: for oxygen consumption of *Arbacia* eggs (Loeb and Wasteneys), oxygen utilization of *Tenebrio* pupæ (Krogh), oxygen consumption of goldfish and of toad (Krogh),

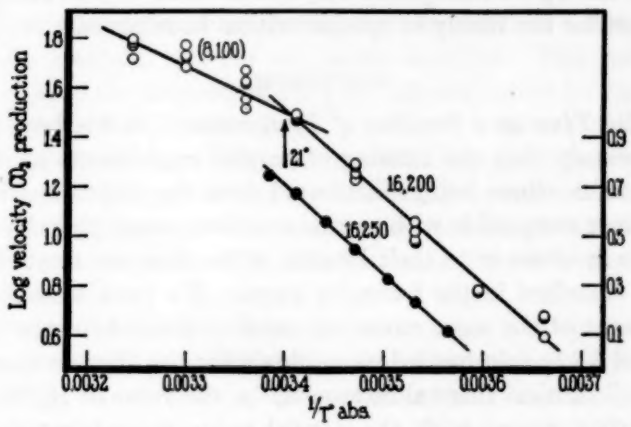

FIG. 4. CO_2 production by seedlings of *Pisum* (Kuyper) reduced to mg. per 100 gm. of seeds, per hour (open circles), and of *Vicia faba* reduced to mg. excreted by single seeds of 5.65 gm., per hour (solid circles).

where we get values from 16,100 to 16,800 (Crozier, 1924). Other instances since secured and collected have brought out this very constant value of μ for a large number of phenomena of that order. If the data of Kuyper are treated in the same way (Fig. 4) we find that they too give a value of μ around 16,200 for the lower range of temperature (up to 20°).[6] Above that temperature we see a new level

[6] The small discrepancy between this value and the one (16,500) published by Crozier (1924–25, *c*) is due to the fact that in the latter case, sets of data relative to each of the first 2 hours were plotted separately. For the present graph, on the contrary, all the data obtainable were plotted and the value of μ corresponds to the slope of the best fitting line for the whole.

being established with a different value of μ, just as is indicated in our case. It must be noted that in Kuyper's as well as in our data the critical temperature, determined by the intersection of the two best fitting lines, gives practically the same point on the thermometric scale, namely 21°C. A much larger departure between these values would not be disconcerting if we consider the heterogeneity of origin and technique in these different series of data. Their fair agreement points out once more that the values are not due to a mere chance distribution but on the contrary to a basic identity of the processes. We hold this very striking similarity of the values of μ as being highly significant for the theory of specific critical increments.

GEOTROPISM.

Reaction Time as a Function of Temperature.—It has been pointed out previously that the running of parallel experiments at different temperatures—these being distributed over the thermometric scale, in the range compatible with normal reactions—may give clues to the processes involved or to their kinetics, if the data are treated by the method described in the foregoing pages. We have seen that such a treatment of the mere numerical results obtained for the CO_2 excretion of *Vicia faba* has led us to obtain for the "temperature characteristic" (critical thermal increment)[7] μ, the value of 16,250, which is in perfect accord with the normal value for respiratory oxidative processes.

In the same way, can we find some other indications which may lead to a view of the mechanism of geotropic curvature? Since a very long time, different authors who have treated this question have considered that two numerical time relations could be determined for such tropistic reactions. The first is the *presentation time*; the second, the *reaction time*. Text-books give generally the following definitions of these two terms (*cf.* for instance Benecke-Jost: *Pflanzenphysiologie*, 1923, ii, 263): *Presentation time* is the shortest time of exposure to the gravitational vector which induces a definite reaction detectable with the naked eye; *Reaction time* is the interval of time elapsing be-

[7] The term "temperature characteristic" was introduced by Crozier (1924–25, *b*) to designate μ without theoretical implications, but this quantity has the dimensions of a critical thermal increment (calories per degree per gram molecule).

tween the moment when the stimulus is applied and the first reaction perceptible to the naked eye. It is apparent from these definitions that these time relations are susceptible to determination only in an approximate manner. But in any case, as we shall see, it is possible to obtain, under standard given conditions, results easily duplicated. In fact, the time of the first reaction observable depends naturally on the magnification of the picture of the organ considered. But one can take as a good indication the much better determined moment where the symmetry of the tip of the root, for instance, ceases and gives place to asymmetry. Furthermore, it should be noted also that these two factors seem to be correlated by Tröndle's rule in a definite and practically constant way for any given material. This rule states that if we vary the intensity of the gravitational vector (or its equivalent and easier handled centrifugal vector) we have a constant relation of hyperbolic form between presentation and reaction times. This is one of the reasons why we can consider presentation time as well as reaction time and even assume than the mechanisms involved in both relations may be very closely similar if not identical at *constant temperature.*

Let us note here also, as a general remark about all geotropic and phototropic experiments with plants, that what we are studying while investigating the reaction of the organism is always a lag period. In fact the chief effect we are following is the mechanism of bending and not directly the first part of the reaction resulting as an effect of a stimulating agency. We can perfectly well conceive that the organism considered in any field of excitation is instantaneously influenced by that field and that a certain "reaction" is started. That this "reaction" has to go on for a certain length of time is indicated by the lag period for the appearance of any external visible sign (*e.g.* a curvature) of "reaction." This curvature may be started only when the "reaction" has persisted for a given length of time or only when certain products determined by the "reaction" have been accumulated up to a certain level. And what we consider then habitually as a measure of geotropic reaction may be an expression of but one step in a chain of coupled reactions, but surely not the first one. This may also be why we can get for geotropic and for phototropic presentation times the same values for μ (*cf.* Crozier, 1924): that value of μ corresponding to

the initiation of the cellular work of bending an organ previously straight. To investigate the primary effects of light and of gravity as means of excitation, in relation to temperature, it would be necessary to introduce an additional variable (such as *intensity*). In the material studied by Castle (1927–28, 1928-29) for example, the temperature characteristics for velocity of elongation (growth) are not the same in different individuals; but at constant temperature the latent period of photic response (a growth elongation) is remarkably constant among a population of individuals.

The number of publications where the relation of presentation or reaction times to temperature has been considered is relatively small. The first data, given by Czapek (1895) and Bach (1907), are of a rather crude nature and seem to have been determined by a method which was unable to yield any better results. The manner of ascertaining the actual duration is open to severe criticism.

In 1912 Maillefer published a series of measurements which are remarkable for the careful determination of the reaction time in *Avena*. Their analysis will be given later. (This paper seems to have been quite overlooked by several authors.) The work of Rutgers (1912) was also executed very precisely; it is concerned mainly with geotropic presentation time in *Avena* and gives the best set of data existing for that quantity. These two papers can be used with confidence as the figures can be duplicated and have all possible reliability. The work of von Paál (1915) and of Tröndle (1915) on the effect of temperature on the geotropic reaction time is not usable, the control of temperature for instance being absolutely too vague. In von Paál's work, for instance, the temperatures are given under the form 22° to 23°, or 12° to 13°, and although experiments have been made at different temperatures his data can not be easily used. In Tröndle's work, the same lack of precision in the control of temperature prevails, and furthermore most of the experiments were performed in the range 18.5° to 25°C. which is too small to give precise indications and is in part *above* the critical temperature.

We are thus reduced to the two sets of data given by Maillefer and by Rutgers. For that reason also, we have tried to determine for the root of *Vicia faba* a series of reaction times which could be compared with the published tables.

The method was a standard one, used by Maillefer and by Rutgers, with the slight modification that the number of plants used was small for each set of experiments at any given temperature, but that the same sets were done several times under the same conditions. The figures obtained in successive runs agree so well one with another as to enable us to use them all together in one table. The variability in response described by von Paál, which in his account was said to become smaller and smaller as the experiment was performed at a temperature closer to the "optimum" temperature for the geotropic reaction, was not found at all in our series. It should be noted, also, that this

TABLE V.

Values of the Geotropic Reaction Time (R.T.) of Vicia faba Roots at Different Temperatures. Data from Czapek (1895), Bach (1907) and from the Present Experiments.

t	R. T.	Czapek's data	Bach's data	*t*	R. T.	Czapek's data	Bach's data
°C.	*min.*	*min.*	*min.*	°C.	*min.*	*min.*	*min.*
5	360*	360		17			115.4
7.5	190			20	57	80	97.9
10	150	120		22.5	51		
12	130			25	51	80	64.8
14			122.8	30		70	48.2
15	100	80					

* This R.T. may be too long; we have only three series of experiments for this temperature.

was not true in the experiments of Maillefer or of Rutgers either. The variability to which Maillefer refers is probably related to a lack of homogeneity in the strain of seeds used.

From this point of view, the material used in these experiments was remarkably constant in its reaction. All the plants used were always chosen to be as similar as possible one to another. The same thing has been found also in series of experiments where the reaction of the *stem* was considered. Table V shows in brief the mean durations of reaction time found for the different experiments.

In the third and fourth columns we have included the tables of Czapek and Bach, as they were the only other figures obtainable for

the same organism. Table VI contains the condensed tables of figures due to Maillefer and to Rutgers (experiments on *Avena*).

Maillefer does not seem to draw any conclusions from his observations, except as to the form of an equation describing the geotropic curvature, which he shows to be a parabola at least for the 1st hour of reaction. On the other hand Rutgers concludes that there is a non-direct relationship between reaction time and temperature, at least for the range 10° to 35°. He calculates Q_{10} and shows it to vary between 0.0064 (for high temperatures) and 6.8 (for the lower part of the thermometric range compatible with geotropic reaction). Between 5° and 35° he finds a $Q_{10} = 2.6$. He is thus in disagreement with Bach but not with Czapek.

TABLE VI.

Values of Geotropic Reaction Time (R.T.) (*Maillefer's Data*) *and of Geotropic Presentation Time* (P.T.) (*Rutgers' Data*) *for Avena sativa, at Different Temperatures.*

t	R. T. (Maillefer)	*t*	R. T. (Maillefer)	*t*	P. T. (Rutgers)
°C.	*min.*	°C.	*min.*	°C.	*min.*
15	72.5	22	37.5	0	72
16	61.2	23	35.8	5	16
17	58.3	24	30.2	10	10.66
18	51.3	25	35.8	15	6
19	46.6	26	36.2	20	4.33
20	41.7	27	30.0	25	2.33
21	40.3			30	3.50

All these conclusions seem to be very different one from another, even often in opposition one with another. Still, taking the data as they are published, it may nevertheless be possible to use them in certain ways. Let us apply to them the same treatment used for the data in the first part of this paper. We find (Figs. 5 and 6) the very striking result that the figures given by the different authors agree very well one with another as to the value of μ. For the different sets we find the following values: Maillefer, 16,200; Rutgers, 16,200; for my personal data, 16,110. All these figures obviously are within the normal range of variations that we may expect for such a value of μ by this

method of computation. It should be noted also that the first departures from the best fitting lines occur practically always at the same temperature: 21.0°, temperature that we found also to be a critical temperature for respiration in this form. We may note furthermore that 5° seems to be a second critical point, although not so well marked; but one must not forget that our observations at that temperature are few. These findings point to the same conclusion: the

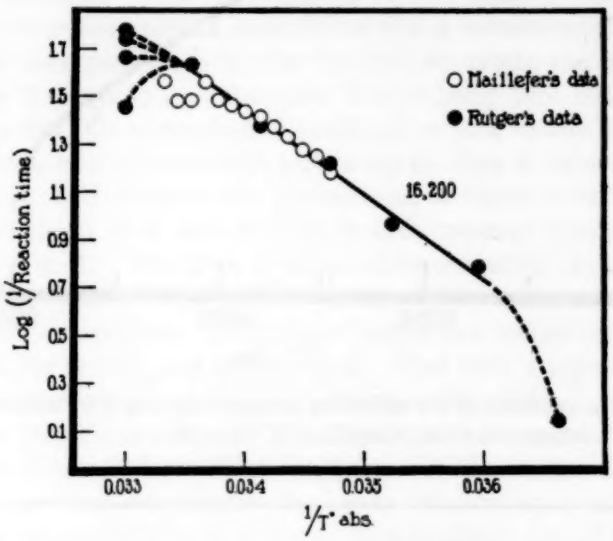

FIG. 5. The velocities of activation processes occupying the "presentation time" (Rutgers' data) and the "reaction time" (Maillefer's data) for geotropic response in *Avena*. The line corresponding to $\mu = 16{,}200$ fits both series with good agreement.

geotropic reaction in the coleoptile of *Avena* or in the root of seedlings of *Vicia faba* gives the same critical increment, 16,200, for reaction or for presentation times. This increment is known to occur in relation to a very large number of processes of respiratory oxidation and in our material also (*cf.* Crozier, 1924). It is then fair to conclude that in the processes underlying the geotropic curvature, a respiratory oxidative effect seems to be the master reaction determining the system of coupled reactions involved.

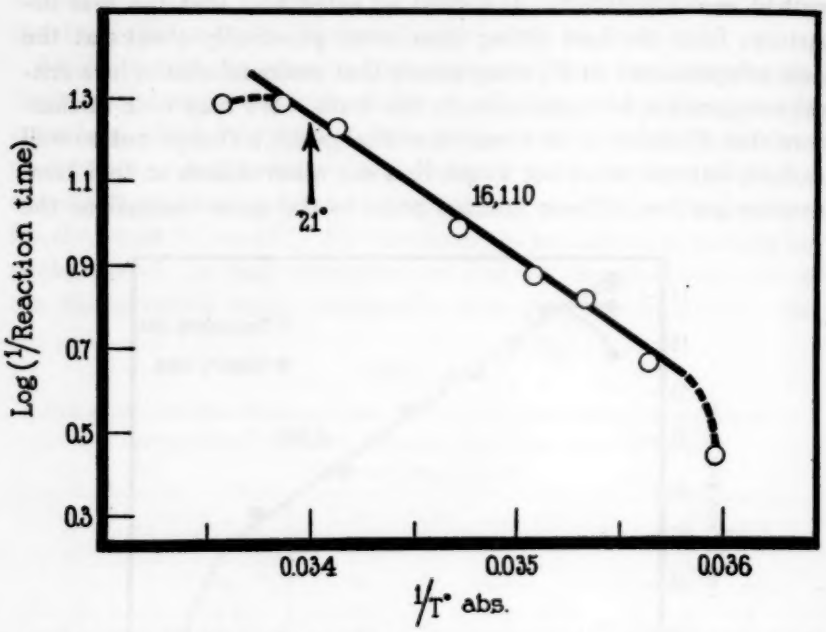

FIG. 6. The velocities of the activation process occupying the "reaction time" for geotropic response in roots of seedlings of *Vicia faba*.

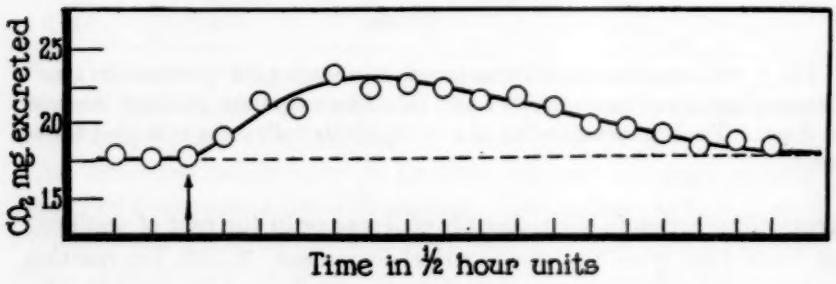

FIG. 7. Curve showing the increased CO_2 excretion when a seedling of *Vicia faba* is geotropically excited. The normal resting excretion is practically at a constant level; the arrow indicates the moment when the plant is turned through 90° and thus geotropically excited.

What this oxidative process is, is yet unknown; further experiments are necessary to ascertain the phases of that reaction; but that such a reaction is occurring, is within the range of certitude of our method of analysis.

An important fact which corroborates this view is the increased respiratory rate that one gets when a plant which has been treated so as to exclude in all prehandling, at any moment, the introduction of the gravitational vector, is subjected to this factor. Such a plant has for the resting rate of excretion of CO_2 a certain constant value even for long periods; if at any moment we rotate the respiration chamber through 90°, all the other factors being kept constant, we find that the rate of respiration increases up to a certain point, and later decreases and comes back after a certain time to its normal resting value. Such a curve, the typical form of which is retained with modifications of scale and of shape at each constant temperature, is given in Fig. 7. This gives a means of investigating the *amount* of geotropic response. The relation between this type of accelerated excretion consequent upon geotropic excitation and temperature will be found in the second part of this work. That such a type of change occurs is perfectly consistent with the values found for μ, for the reaction or presentation times. This may in part also explain why most of the authors who have determined the rate of excretion of CO_2 by seedlings got variable results: they were determining a mixed rate, perfectly uncontrollable, of CO_2 excretion in plants placed in all the intermediate positions between a normal resting state and a highly geotropically excited state. It can be added also that the temperature may even not have been under effective control, in the cases where the bulb of the thermometer was placed in the mass of seeds. This shows the advantage of the method where one seedling alone is used for each experiment and all the factors are under control.

SUMMARY.

In this paper there are given the results of a study of the relation of respiration to temperature, in seedlings of *Vicia faba*, and of the onset of geotropic response. It is shown that with due care and very accurate control of temperature constancy, one can get constant ex-

cretion of CO_2 even over fairly long periods. The treatment of the experimental data shows that the Q_{10} ratio is of course a valueless "constant," as it is variable; but that the figure obtained for μ, the temperature characteristic (critical thermal increment), 16,250, is perfectly consistent with the values previously obtained for μ in respiratory oxidative processes in similar material. New data on the reaction time for the root of *Vicia faba* seedlings excited geotropically are given also. The study of the dependence of this time relation on temperature shows μ = 16,110, agreeing quantitatively with the value deduced previously from the relevant data of earlier investigators (Crozier, 1924). This points to the importance of some respiratory oxidative process as the agency controlling the onset of geotropic curvature.

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THE PHOTOTROPIC EXCITATION OF LIMAX.

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I.

From measurements of the circus movements of *Limax* as governed by intensity of illumination it was deduced (Crozier and Cole; *cf.* Crozier, 1928) that over the range of intensities employed the mean amplitude of the orienting movement (degrees turned *per* centimeter of path) is directly proportional to the logarithm of the intensity. By a quite different method it was found (Wolf and Crozier, 1927–28) that with *Agriolimax*, which becomes much more quickly adapted by light, the velocity of photic adaptation is proportional to the logarithm of the intensity, as well as showing a logarithmic decrease in excitation with time of exposure to light of constant intensity.

The method employed with *Agriolimax* consisted in having the slug creep upon a vertical plate with the constant orienting effect of gravity continuously opposed by the action of a horizontal beam of light. The effect of the photic excitation is then to induce locomotion not straight upward, as in darkness, but at an angle β to the vertical away from the light. The course of light adaptation and of dark adaptation as measured by the changes in the magnitude of this angle can be fully formulated (Crozier and Wolf, 1927–28; 1928–29) on the assumption that the excitation is at any moment proportional to the rate of photolysis of sensitive material S, undergoing a first order decomposition by light and reconstituted by a "dark" reaction which is second order and with positive autocatalysis. With ordinary intensities of illumination, the course of the decomposition of S in the system

$$S \underset{\text{"dark"}}{\overset{\text{light}}{\rightleftharpoons}} P + A$$

$$\| S \|$$

where $||S||$ indicates catalysis by S, is accordingly given by

$$-\frac{dS_i}{dt} = K_1 I S_i - (K_2 + K_3 S_i)(S_\infty - S_i)^2,$$

where S_∞ = the maximum amount of S which may be present at complete dark adaptation. Since K_1 is much greater than the other velocity constants (that is, light adaptation is here relatively much swifter than the reverse process of dark adaptation) the velocity of the light adaptation, under conditions imposed by threshold relations which must be satisfied in order to overcome the geotropic effect and thus to obtain any photic orientation, will appear very nearly proportional to *log I*, as already found (Wolf and Crozier, 1927–28). In the case of a similar organism which becomes only very slowly adapted by the light it may be supposed that the rate of photolysis of S is actually very slow, in terms of the total amount of change possible, or that sensitive material is in some way supplied by an independent mechanism. Other possibilities may be appealed to, as related to the absorption of light in the sensitive cells, but in any case we might still expect that excitation would be nearly proportional to *log I* over such intermediate ranges of intensity as alone are open to test (*cf.* Hecht, 1919–20; 1922–23).

We have examined the behavior of *Limax* creeping upon a vertical plate with lateral illumination, to contrast its responses with those of *Agriolimax*, because its rate of light adaptation is much slower than with the latter form. From Yagi's (1927–28) experiments with *Dixippus* we already have evidence of this kind for the proportionality of photic excitation and *log I* in a form which adapts only slowly to light. We desire to see, however, if the conclusion regarding *Limax* as based on the analysis of circus movements could be confirmed by this independent method.

II.

As a matter of convenience it has been usual to deal with fields of excitation which are geometrically simple. For the development of the method of tropistic analysis, however, it is required to examine more complex cases (*cf.* Crozier and Stier, 1928–29; Buder, 1917–19; Mitchell and Crozier, 1927–28). This is particularly necessary when

considering the compounding of tropistic effects (Crozier and Pincus, 1926–27; Crozier and Stier, 1927–28; Wolf and Crozier, 1927–28). It

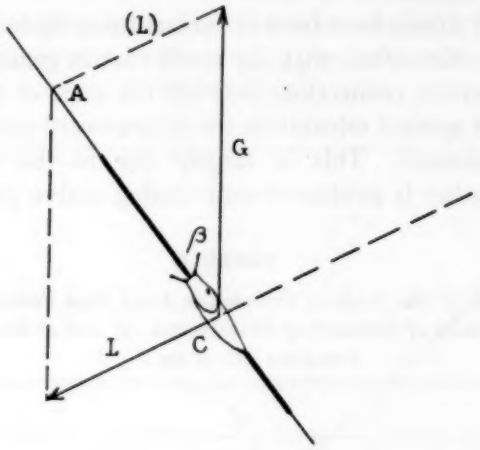

FIG. 1. A slug (*Limax*), previously dark-adapted, is creeping on a vertical plate of ground glass which has been moistened. It is illuminated at an angle from one side by light from a small condensed filament lamp. To avoid effects of the observer's breathing currents, the path of the slug is observed from the other side of the plate. At the point C the slug is under the influence of an orienting field containing two significant vectors: a gravitational vector G, which is assumed constant; and a photic vector L, of magnitude assumed proportional to *log* I. I is *measured* at C, with a photometer giving illumination in foot candles. The direction of progression is given by the heavy line b. Then if the assumptions are valid

$$L = K \log I = \left(\frac{G}{\sin A}\right) \times (\sin \beta)$$

or,

$$\frac{K' \sin \beta}{\sin A} = \log I \qquad (2)$$

The plot in Fig. 2 shows that the equation is satisfactorily obeyed.

becomes desirable to formulate orienting movements when the competing excitations do not act at right angles or directly in opposition (Crozier, 1926–28). An elementary instance is given by the following

series of experiments, in which the difference from those cited in the previous section lies in the angular inclination of the photic and the gravitational vectors.

Numbers of *Limax* have been at various times thoroughly examined for geotropic orientation, with the result that in general it is difficult to obtain clear-cut connections between the slope of the surface and the amount of upward orientation (or of downward orientation during geopositive phases). This is largely due to the fact that geotropic orientation is evidenced only during active progression, and

TABLE I.

Observed Values of the Angle of Orientation Away from Vertical (β), at Various Magnitudes of Intensity of Illumination (I) and of the Angle of Incidence (A) of the Light.

I	A	β
foot candles	*degrees*	*degrees*
2.6	98.8	41.4 ±3.4
2.5	101.6	36.8 ±4.9
2.3	96.4	37.8 ±5.4
1.53	87.6	33.0 ±4.5
1.25	90.0	33.1 ±4.0
0.96	84.6	27.9 ±4.1
0.77	90.0	25.1 ±3.6
0.57	82.9	23.5 ±3.7
0.52	83.5	22.0 ±4.6
0.48	86.1	18.8 ±3.1

is effected by the speed of progression, within certain limits (*cf.* Crozier, 1928). We have found it better, therefore, for the present purpose to utilize groups of young *Limax* hatched from egg masses in the Laboratory and raised to a length of approximately 1 cm., being fed on decaying wood, and apple and the like. Such individuals are occasionally indifferent to light (*cf.* Crozier and Libby, 1924–25; Crozier and Federighi, 1924–25). Provisional testings are therefore required for the selection of sensitive individuals. So far as possible such individuals were used in a series of tests at several intensities. The animals were handled as in the experiments described by Wolf (1926–27) and Wolf and Crozier (1927–28; 1928-29).

The conditions in the tests are given diagrammatically in Fig. 1. The measured angles are β and A; O is the mid-point of the oriented trail on the vertical plate. Then, G being assumed constant, and if L be proportional to $\log I$, the condition which should be obeyed is:

$$\log I = \frac{K \sin \beta}{\sin A} \tag{1}$$

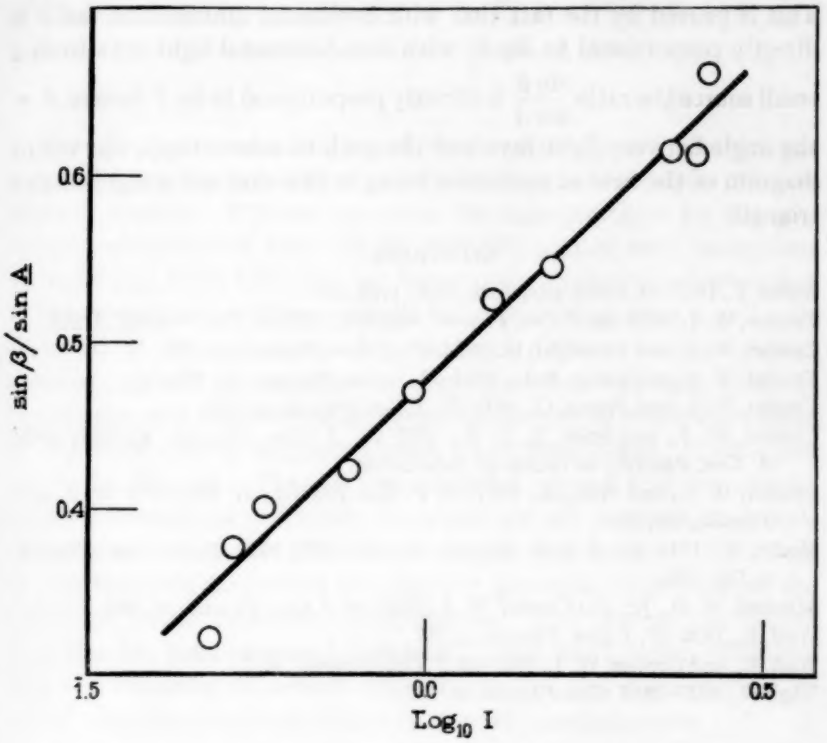

FIG. 2. Log I plotted against sin β/sin A (*cf.* Fig. 1) shows a rectilinear relationship. Data in Table I. The residual variation (Table I) is approximately constant throughout (*ca.* $\pm$ 4.0°).

The data are given in Table I, and are plotted in Fig. 2. The agreement must be considered very good. The deviation at the lowest intensity is fully covered by the probable error, and in fact a positive error of 1.2° of arc in the mean value of β (easily possible in view of

the inevitable crudity of the measurements) would bring this point back on the line fitted to the observations.

SUMMARY.

The photic orientation of *Limax* creeping geotropically upon a vertical plate is such that the phototropic vector determining the angular deflection β from the vertical path is proportional to *log I*. This is proved by the fact that with horizontal illumination tan β is directly proportional to *log I;* with non-horizontal light rays from a small source the ratio $\frac{\sin \beta}{\sin A}$ is directly proportional to *log I* (where A = the angle between light rays and the path of orientation), the vector diagram of the field of excitation being in this case not a right-angled triangle.

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GEOTROPIC ORIENTATION IN ARTHROPODS.

II. TETRAOPES.

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I.

The geotropism of certain arthropods offers opportunity for the evaluation of descriptive equations derived from the behavior of forms already studied. If these equations, for example those for the geotropic orientation of slugs (Wolf, 1926–27) and of tent caterpillars (Crozier and Stier, 1927–28), are based upon a correct understanding of the mechanisms which essentially limit orientation, then a geotropic organism of different morphology, or with different method of progression, should be expected to obey somewhat different rules, in correlation with any obvious difference in the way in which the weight of the body can be supported upon an inclined plane. This turns out to be the case. There is thus provided a proof that the quantitative formulations previously discussed are not mathematical accidents in some way due to the averaging of observations. This kind of test has been made with the negative geotropic orientation of the milkweed beetle *Tetraopes tetraopthalmus*. The case of the fiddler crab *Uca* has been examined elsewhere (Kropp and Crozier, 1928–29), and the behavior of several other forms has also been studied in detail; the latter instances will be discussed in another place.

II.

Tetraopes was collected on milkweed plants and kept in the laboratory in terraria upon transplanted milkweeds. The animals are hardy, and submit in a satisfactory way to a variety of manipulative operations. Their geotropism is normally precise and negative. The oriented upward progress is however periodically interrupted by brief intervals of cessation of creeping, even upon a vertical surface, which

may be accompanied by more or less vague turning movements, and at times by transient but definite *positive* orientation. Here, as in other instances, there accordingly appears an important correlation between continuity of progression and precision of geotropic orientation. Moreover, the equilibrium angle of orientation, θ, also varies, within certain limits, and with fairly definite rhythm, as already mentioned for caterpillars (Crozier and Stier, 1927–28). It is notable that in the cases of geotropism with which these experiments have been concerned the assumption of a significant alignment of the axis of the organism upon the plane (as defined by the angle θ) is apparent only *during progression*. A similar relationship between orientation and creeping is evident in experiments upon a turntable. It may be taken to mean that the central nervous condition attending the rhythmic use of the appendages (or, with caterpillars, perhaps the body musculature also) in creeping permits the effect of tensions applied to the muscles to exert an influence upon orientation. This may be due, however, to the fact that *change* (increase) of tension is the effective stimulus for the tension receptors, as is true in vertebrates. Interesting questions arising from such considerations, and in connection with the intermittent failure to maintain orientation, we must put aside for the present with this bare notice of their realization.

The geotropism of *Tetraopes* is very strikingly affected as a result of mechanical stimulation such as that due to handling. In this respect there is a certain parallelism with the reversals of phototropism seen in various animals as a result of mechanical excitation. The method used to obtain geotropic trails avoided any such disturbance. As the beetle approached the upper edge of the sheet of white paper on which it was creeping upward, a small piece of paper held at one corner by the experimenter was placed in its path; when the beetle had mounted it, the paper was moved to the lower edge of the recording sheet, where, after suitable rotation of the carrier, the animal was allowed to creep back again upon the record sheet. The rotation was necessary to enable the new trail to be initiated with a path almost horizontal in direction, from which upward orientation followed. The absence of any special effect due to handling is attested by the uniformity of the results as gotten by each of the two observers.

Trails were also obtained by the method earlier described for tent

caterpillars, in which a platform 3 meters long permitted very extensive creeping. In this way it was possible to study spontaneous changes of the angle θ, and the periodic halts and other phenomena associated with such shifts.

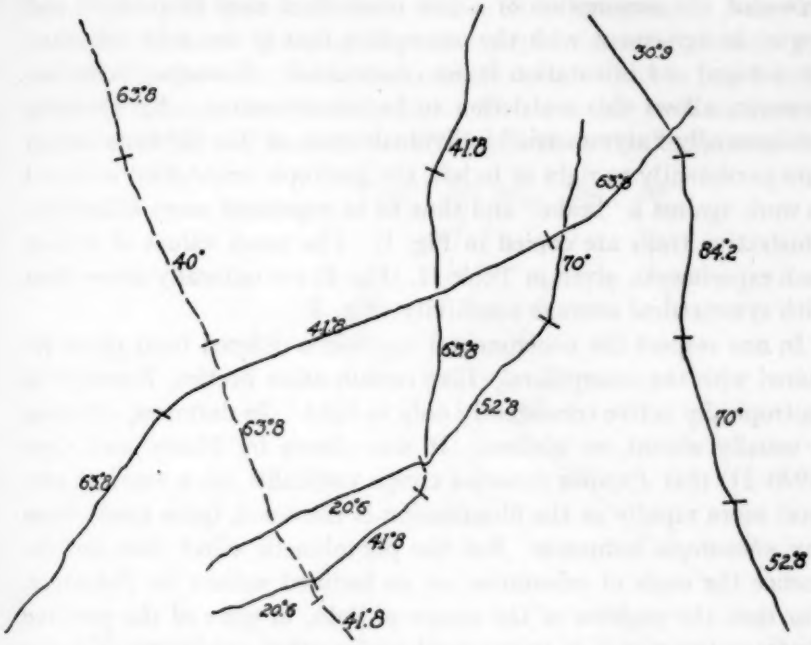

FIG. 1. Tracings of trails, selected at random, to show dependence of θ, the angle of upward orientation, upon the indicated tilt (α) of the plane upon which *Tetraopes* is creeping. Individuals temporarily asymmetric in response, *i.e.*, tending persistently to turn to right or to left, were purposely chosen for these tests in which α was altered (increased or decreased) without touching the beetles; for this reason the magnitudes of α are lower than those obtained as general averages (*cf.* Table I, Table II, and text).

It is desirable to realize that the dependence of the angle θ upon the inclination α can be demonstrated by a technic which avoids any possible consequence of immediate handling of the animals, and which is largely unaffected by spontaneous fluctuations in geotropic sensitivity. (This particular procedure, also employed by Crozier and Pincus (1927–28) in experiments with rodents, not yet described, has

the advantage that it permits a subsequent study of the latent period for geotropic response.) It consists in tilting the creeping plane at various angles, during progression of the animal upon it. The method has with some forms a certain restriction. If the inclination be *decreased*, the assumption of a new orientation may be delayed and vague, in agreement with the conception that in the field delimited by $+\theta$ and $-\theta$ orientation is not constrained. *Tetraopes'* behavior, however, allows this restriction to be circumvented. By choosing pronouncedly "asymmetric" individuals such as (for the time being) turn persistently to right or to left, the geotropic orientation is forced to work against a "brake" and thus to be expressed more delicately. Illustrative trails are copied in Fig. 1. The mean values of θ from such experiments, given in Table II, (Fig. 4) are naturally lower than with symmetrical average sensitivity (Fig. 3).

In one respect the experimental conditions differed from those required with the caterpillars. Like certain other beetles, *Tetraopes* is geotropically active consistently only in light. In darkness, creeping is usually absent, or aimless. It was shown by Moore and Cole (1920–21) that *Popillia japonica* creeps vertically (on a vertical surface) more rapidly as the illumination is increased, quite apart from any *phototropic* influence. But the photokinetic effect does not influence the *angle* of orientation on an inclined surface, in *Tetraopes*. Nor does the position of the source of light, in spite of the positive phototropism which is pronounced under other conditions (Crozier and Mangelsdorf, 1923–24). Our experiments, however, were made in a dark room with light (and diffusing screen) directly above the creeping stage, of 40 m.c. intensity. With such illuminations, light may come either from below, ahead, or from one side of the animal without modifying θ. With light of much lower intensities it is apparently possible to compound positive phototropic and negative phototropic orientations upon inclined surfaces, but the very interesting additive and threshold relationships between vectorial phototropism, geotropism, and photokinesis we have not as yet been able to examine in detail. Occasionally, good creeping was had without high light intensity. One such instance was given by beetle 16. With $\alpha = 49°$, trails under white light gave $\theta = 74.48°$; under weak red light, $\theta = 74.60°$. In subsequent experiments with *Pyrophorus* (Kropp, 1929) observations could be made in darkness.

The absence of effective directional action by light of higher intensities in these experiments has one fortunate consequence. The use of rough white paper as a creeping surface did not introduce errors due to orientation by reflected light. This was checked by comparison with trails upon dull black cloth. It is nevertheless true that when in geotropic creeping on white paper the beetle closely approaches the boundary of a black surface upon which the paper rests, the trail suffers noticeable deflection. But it is only such contrast as between white and black areas which produces this temporary dis-

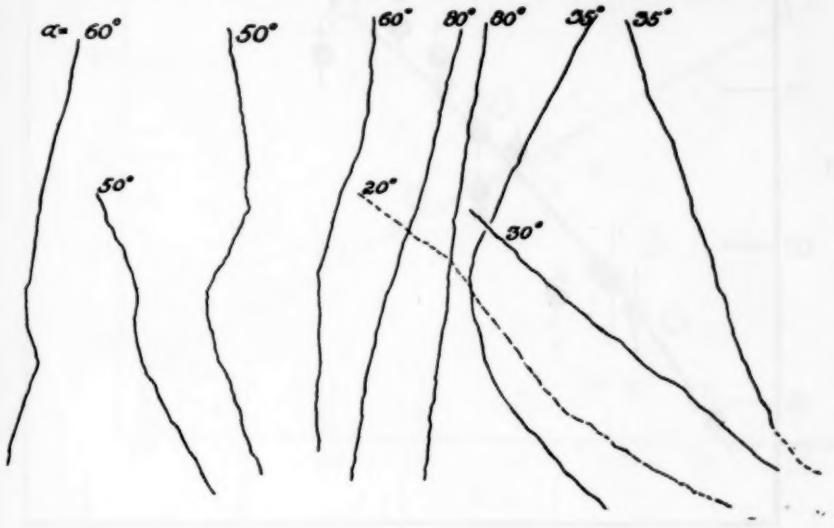

FIG. 2. Various trails, chosen at random, to illustrate properties of the records of orientation of *Tetraopes* on inclined surfaces; see text.

turbance, and even then only when the beetle is creeping slowly but not (as a rule) when moving with optimum speed.

In the field, flying of the beetles is notable on bright sunny days with high temperature. At 25° to 27° a flying beetle lighting upon a milkweed immediately creeps upward and usually flies off on reaching the top. This happens at once upon arrival at the highest point of support if there is apparent coincidence with one of the rhythmically recurrent spasms of separation of the elytra and fluttering of the wings. With higher temperatures (>27°) these periods are much

more frequent. This was also studied with beetles enclosed in a large spherical flask immersed in a thermostat at controlled temperatures. The spasms of elytral opening occur at longer intervals at 24°, although

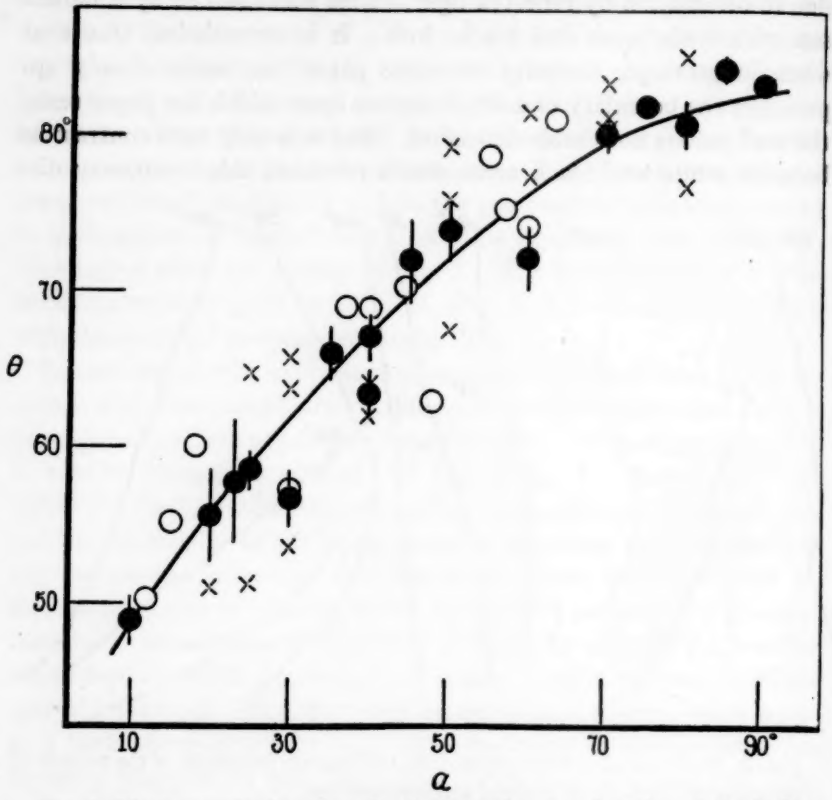

FIG. 3. Mean magnitudes of angle of orientation θ as function of the inclination of the surface α. Values which are averages from fewer than ten series of measurements are given as open circles, of ten or more as solid circles (with the probable error of the mean of the series' means indicated). There are added (crosses) mean estimates of θ from tests with a single individual (No. 15) used at a large number of inclinations. The curve is that of $\Delta\ \theta / \Delta \sin \alpha = const.$ (*cf.* Fig. 7).

even here an activity of this kind may *rarely* eventuate in flying departure from even a flat surface. To obtain creeping uninterrupted by these disturbances, the geotropism tests were made at temperatures between 23.5° and 25°.

No differences in orientation were observed which could be correlated with sex or size of individual, and the results from all have therefore been taken together. Between 10 and 20 trails were secured, at various angles of inclination of the supporting plane, with each of some 56 individuals. Not all of these were used at every inclination, some being chiefly employed for operative experiments.

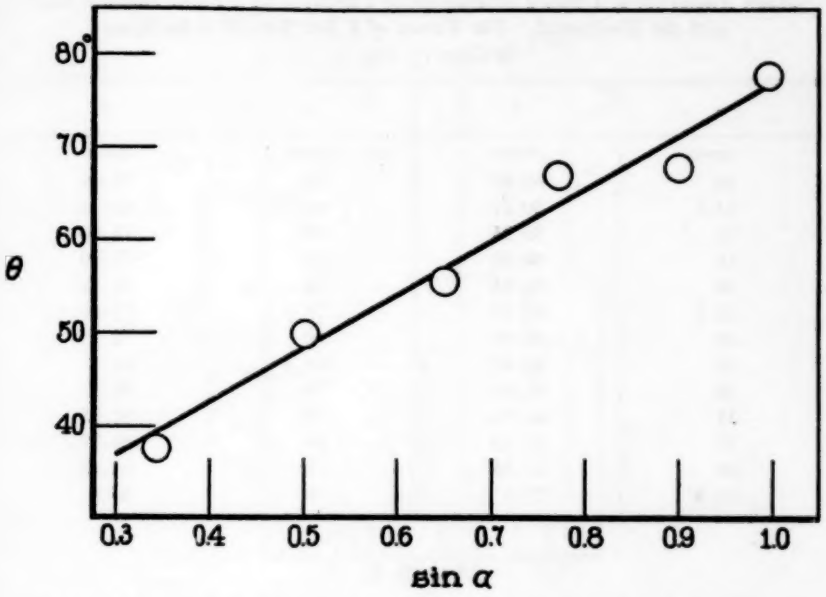

FIG. 4. Mean values of θ as a function of sin α, from trails with 7 markedly asymmetric individuals (*cf.* Fig. 1, and text). The values of θ (Table II) are lower than the averages at corresponding inclinations with normal (*i.e.* not asymmetric) individuals.

A total of approximately 3,400 trails form the basis of Fig. 3. The variability of θ, under these conditions, cannot be expected to show the sort of regularity discovered in other experiments with groups of selected individuals, and it is significant that the variation of the mean θ's as tabulated cannot be reduced to any simple rule. The angles of orientation (θ) were measured upon the trails as accurately recorded with soft pencil during the progress of creeping. Illustrative details are given in Fig. 2. As with the caterpillars, there occur periods of

marked asymmetry of response. During such a period it is sometimes impossible for several hours to obtain a single trail with orientation to the right (or to the left, as the case may be) but all go to the opposite side no matter how the beetle is started creeping. Several

TABLE I.

Mean Angles (θ) of Upward Orientation of Tetraopes on a Plane Inclined at $\alpha°$ with the Horizontal. The Values of θ Are Not All of the Same Weight; Cf. Fig. 3.

α	θ	α	θ
degrees	*degrees*	*degrees*	*degrees*
10	48.89	45	71.99
12.7	50.21	46.5	63.13
15	55.25	49	74.60
18	60.02	50	73.99
20	58.04	55	78.78
23.3	62.15	57.1	75.69
25	58.96	60	72.2
28	62.92	63.3	81.17
30	61.64	70	80.17
35	66.04	75	81.95
37	68.63	80	80.77
40	63.50	85	84.35
44.3	70.3	90	83.3

TABLE II.

Mean Angles of Upward Orientation (θ) from Experiments in Which 7 Tetraopes Markedly Asymmetric in Response Were Allowed to Creep upon a Platform the Slope of Which (α) Was Subsequently Altered to the Value Noted (See Text, and Fig. 1).

α.....	20.6°	30.9°	41.8°	52.8°	63.8°	84.2°
θ.....	37.78°	50.00°	55.51°	67.08°	67.99°	77.95°

hours later, or next day, the orientation of this individual may be equally definite but to the other side. We have not attempted, in such cases, to apply the method of averaging used with the tent caterpillars, but have simply taken the values of θ, in view of the absence of any systematic divergence. Creeping is slower at higher values

of α (and of θ), but this was not studied systematically. In every case the beetles used showed a normal activity of the legs in creeping; it is *particularly* necessary to be sure that the tarsal hooks have not been torn off when the animal is removed from the milkweed, or during subsequent handling. Deficiencies of this kind, or injuries to the

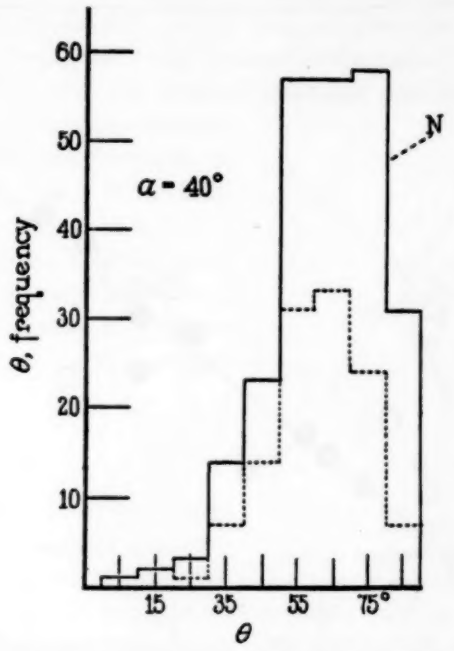

FIG. 5. Distributions of measurements of θ for all observations at $\alpha = 40°$; for normal individuals (N) and for those with antennæ removed.

legs, quickly reveal themselves in irregularities of orientation and in circling movements; the functional significance of the legs differs among the 3 pairs and all 6 legs are required for adequate progression, as proved by a variety of amputation tests.

III.

The average angles of upward orientation (θ) of *Tetraopes* are related to the tilt of the creeping surface as set out in Table I (Fig. 3). Each average is the mean of the averages of θ for each of a number of

individuals. The manner in which the separate readings of θ are distributed about the means is illustrated in Fig. 5. These distributions are essentially symmetrical, at inclinations below $\alpha = 60°$. The proportionate scatter in such graphs is not fairly dealt with unless the size of the θ - classes is made less in proportion to sin α; this is due

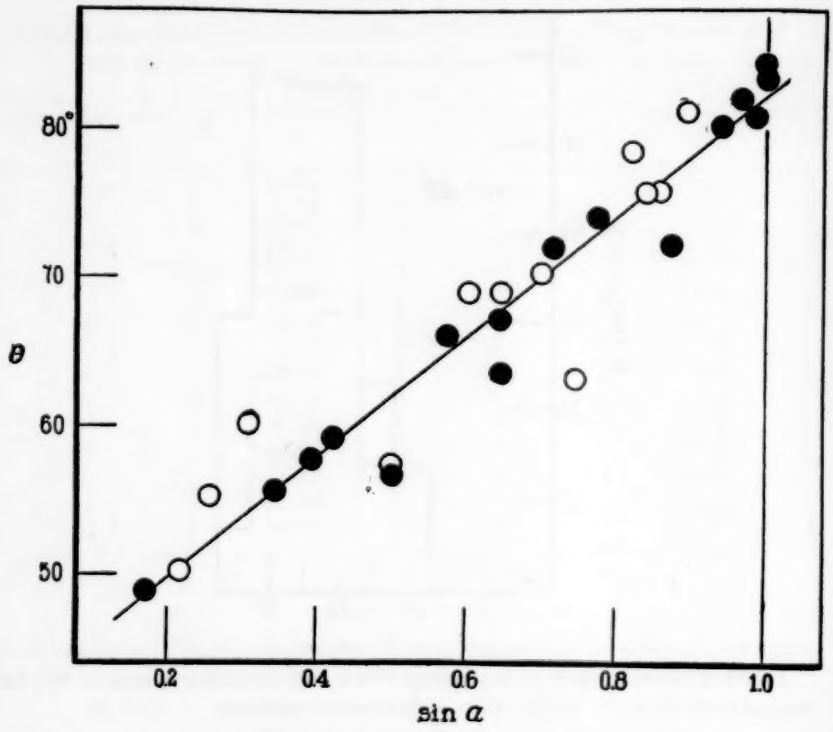

FIG. 6. The relation between θ and sin α is practically linear (*cf.* Fig. 3); weighing of the points is as indicated in Fig. 3.

to the fact, brought out in earlier accounts (Crozier and Pincus, 1926–27, *a*, *b*), that the variability of θ is itself a function of sin α. Such a result provides an important test of the adequacy of the experimental procedures. At high inclinations the distribution of θ is necessarily somewhat skew, since the animal cannot very well give $\theta > 90°$. The situation here as regards the distribution of θ's is pre-

cisely that already described in our preceding paper (Crozier and Stier, 1927–28, *b*). The distribution of the mean values of θ obtained from the several individuals is also "normal." A sufficient method of weighing these means is to give the number of "runs" of observations concerned in each. In Fig. 3 the points based upon averages from fewer than 7 individuals are entered as open circlets.

Examination shows that in the case of *Tetraopes* the angle θ is linearly proportional, not to *log sin* α as in the earlier cases, but to *sin* α directly. The lowest tilt at which *consistent* orientation is obtained is $\alpha = 15°$; below this, positive and negative orientations fluctuate, or creeping is random. With some individuals the threshold is at 20°. With angles above $\alpha = 85°$ creeping is slowed, and no increase in θ is to be detected. Between these limits the ratio $\Delta\theta/\Delta$ *sin* α is constant (Fig. 6).

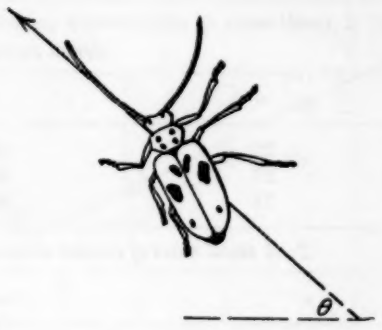

FIG. 7. The posture of *Tetraopes* upon an inclined surface. Owing to the mass of the abdomen, a twisting moment must be supported by the legs when $\theta < 90°$. Diagrammatic.

IV.

Inspection of the creeping beetles at once suggests a possible origin for the difference between the descriptive formula for their orientation and that for the other forms thus far considered. The direct rather than logarithmic relationship between θ and the sine of the inclination of the surface, whatever its mechanism, is incidentally a valuable commentary on the point previously made (Crozier and Pincus, 1926–27, *a*, *b*, *c;* Crozier, 1928; and Crozier and Stier, 1927–28) regarding the "Weber-Fechner Law." In *Tetraopes*, as with certain other beetles, the center of gravity is posterior to the region of attachment of the legs (Fig. 7). This must result in a turning moment applied to the legs, in addition to the straight downward pull which they support parallel to the inclined plane. The same effect is seen in elaterids; of these, several luminous Cuban species were studied be-

cause it is possible to follow their paths in darkness. If the notion be correct that the gravitational pull supported by the legs during creeping supplies the gravitational stimulus, and also ,because of a differential distribution upon the appendages of two sides, provides the limiting condition necessary to establish θ as a function of α, then the relationship should be experimentally controllable by modifying the

TABLE III.

Results of Experiments in Which the Distribution of the Weight, and the Total Load, Carried by Tetraopes during Upward Geotropic Creeping Was Varied.

1. A small mass of colophonium cement weighing 3.8 mg. attached to the posterior dorsal surface of the elytra.

α	θ	θ with wax attached
25°	61.1	68.4
55	80.3	84.6
75	82.0	81.93

2. A small mass of cement attached to the dorsal surface of the thorax.

α	θ	Attached mass	θ with wax attached
25°	61.1	3.8 mg.	57.6°
35	64.5	21.2 mg.	35.4 (90 per cent trails +)
	74.04	3.8	48.6 (30 per cent trails +)
55	80.3	3.8	63.4
75	82.0	3.8	81.2

3. The wing covers and wings removed.

α = 40°; mean θ for the individuals used in these tests = 67.1° ± 1.5° before removal of wings; θ = 69.0° ± 2.0 after removal.
(Removing the antennæ, also, has no detectable effect).

several components of this pull. Removal of the antennæ of *Tetraopes* has no effect on orientation (*cf.* Fig. 5). In land pulmonates (Crozier and Federighi, 1924–25; Cole, 1925–28; Crozier and Navez, 1928–29) and in young mammals (Crozier and Pincus, 1926–27, *a*, *b*; 1927–28; Pincus, 1926–27) control through effect on the tensions of muscles is quite clear; in certain other instances, according to a few tests made, for example, by Fraenkel (1927) with *Littorina*, and

(unpublished) by Mr. E. F. B. Fries with *Dendronotus*, the effect if any is dubious with these particular aquatic forms. The experiments with some forms are hampered by the nature of the creeping mechanism (*Dendronotus*); in the case of others it is first necessary to remove the possible influence of other sources of stimulation, and to make certain that the animal is moving with adequate speed (as has been found with *Littorina* creeping in air). In still other cases, among such pulmonates as *Limax*, for example, comparative measurements can only

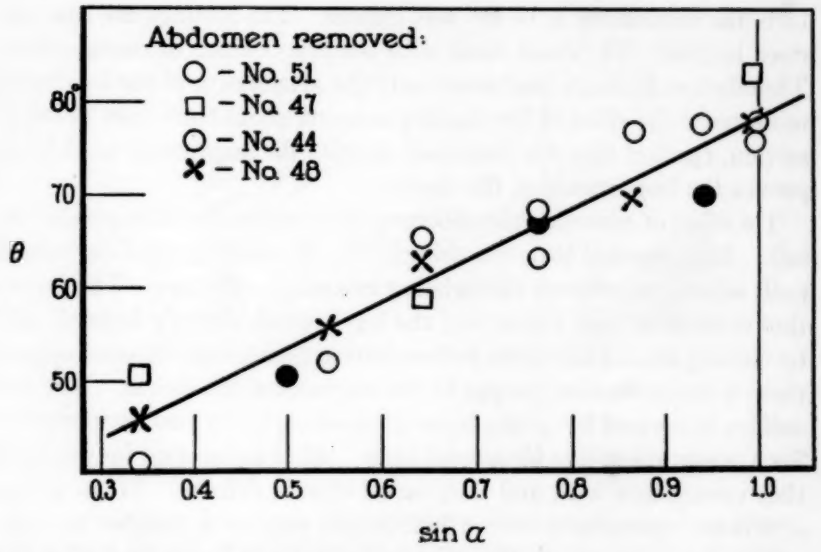

FIG. 8. Mean values of θ at various slopes (α) of the surface on which creeping takes place, for 4 individuals after amputation of the abdomen.

be obtained when the animals are creeping with comparable degrees of extension of the body, since the tonus or the degree of extension of the musculature must be supposed to influence the sensitivity of its proprioceptors to deforming pressures; this rather elementary kind of error (together with several others) has for example led Piéron (1928) distinctly astray, as our own numerous earlier experiments with *Limax maximus* suffice to demonstrate.

With *Tetraopes* it is a simple matter to perform experiments of the following kinds: *1*, an additional mass (a small bit of colophonium

cement) is attached at the posterior margin of the elytra; *2*, a similar addition is made to the head or at the anterior border of the thorax; *3*, the wing covers and wings may be removed; *4*, the whole abdomen may be cut away.

Experiments of type *1* should increase θ, slightly, at constant values of α, if α is not too large; in type *2*, θ should be decreased; in *3*, θ should be very little affected, but if anything decreased; in *4*, it should be markedly decreased. The results obtained in a long series of trials agree with these predictions, in every particular. For most of the tests the inclination $\alpha = 40°$ was chosen. The findings are summarized in Table III, which deals with the first 3 kinds of modification. The effect of adding a load anterior to the attachment of the legs being to decrease the effect of the turning moment about their lines of intersection, the fact that θ is decreased, despite the augmented total load, proves the importance of this factor.

The effect of removing the abdomen must be considered in greater detail. It is expected to do two things: first, to lessen the total downward pull; second, to remove the twisting moment on the legs. The operation is made in such a way that the legs are not directly injured, and by cutting around the chitin before cutting through the internal organs there is less extensive damage to the contents of the thorax. The cut surface is covered by a thin layer of vaseline, to prevent evaporation. Such preparations live for several days. After an interval for recovery they creep fairly well, and their orientation is definite. Mean values of θ from experiments with 4 individuals used at a number of inclinations are collected in Fig. 8; θ is consistently lower than with the intact animals (Fig. 6). In terms of the preceding suggestions, the lower values of θ resulting after amputation of the abdomen should be corrected by attachment of a substitute mass of wax. Thus in the case of *Tetraopes* No. 51:

$\alpha = 35°$
$\theta = 65.0°$
θ, abdomen removed, $= 48.9°$
θ, abdomen removed $= 75.6°$
but with load of
wax (8.9 mg.) attached in its place,

If the turning moment due to the abdomen is responsible for the failure of an approximately logarithmic connection between θ and $\sin \alpha$, then

such beetles should in their orientation obey a formula different from that obtained for the normal *Tetraopes*. Experiments with several active preparations are summarized in Fig. 8. It is apparent that the relation of θ to $\sin \alpha$ is however again direct, as nearly as can be told, rather than logarithmic; the difference must arise in some other way.

These experiments give, then, an additional check upon the theory of geotropic orientation, in two particulars: (1) the different formu-

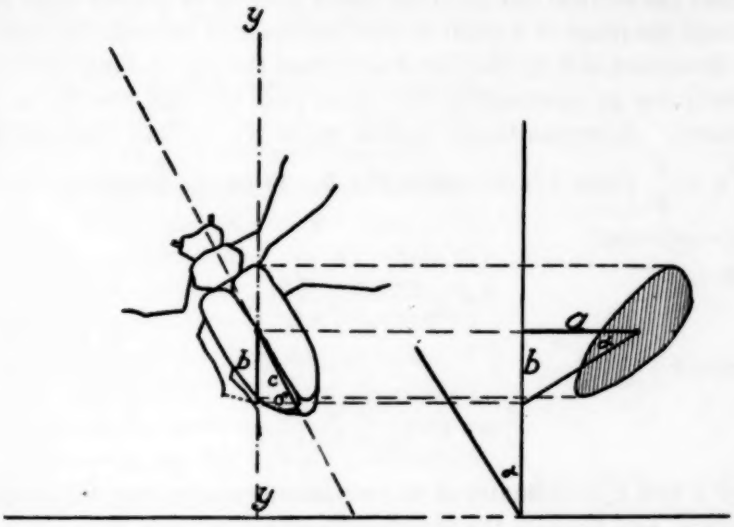

FIG. 9. Projection of a creeping *Tetraopes* upon an inclined surface (diagrammatic), and at the right a vertical section in the plane $y \ldots . y$. The line of gravitational pull passes downward from the center of gravity, and is assumed (as a limiting condition) to pierce the surface on the line connecting the tip of the abdomen, which is in contact with the surface, with the tip of the midmost position of the mesothoracic leg. See text.

lations required according to differences in structure, and (2) a test of the muscle tension theory when the gravitational pull can be, not merely increased as in former experiments, but also decreased as well. It remains to find a rationalization of the quantitative relations in the case of *Tetraopes*. This is done in the following way. Let it be considered that the sideward displacement of the body axis through the pull of the abdomen is for the moment ignored. Then the conditions

of progression may be diagrammed as in Fig. 7 and in Fig. 9, in which the outline of the beetle is projected normally upon the plane of creeping. But in fact the body is effectively supported at 3 points only, namely, by the legs on either side and by the posterior end of the abdomen (it is true that the mandibles are also, at each step, in brief contact with the substratum), and except at these points it is not in contact with the plane. A prime condition for stability, then, will be that the vertical line from the insect's center of gravity must pass through the plane at a point within the "base of support" defined on the downward side by the line drawn from the legs (taking the mesothoracic leg as representing the mean position) and the tip of the abdomen. A section in the vertical plane $y \ldots . y$ then discloses that $\tan \alpha = \frac{b}{a}$, where b is defined in Fig. 9. From the projection on the creeping plane,

$$b = \frac{x \sin (\pi - \delta)}{\operatorname{Sin} (\pi - 90^\circ + \theta - \delta)}$$

therefore

$$\frac{a}{c} \tan \alpha = \frac{\operatorname{Sin} (\pi - \delta)}{\operatorname{Sin} (90^\circ + \theta - \delta)}.$$

Since a and c, coordinates of the center of gravity, may be assumed constant, and likewise the structural angle δ,

$$K \tan \alpha = \frac{1}{\sin (90^\circ + \theta) \cos \delta - \sin \delta \cos (90^\circ + \theta)}$$

$$= \frac{1}{K' \sin \theta + K'' \cos \theta}$$

$$K_1 \cot \alpha = K_2 \sin \theta + \sqrt{1 - K_2^2} \cos \theta.$$

Values for the constants may be gotten by substituting pairs of values of α and θ. A plot of this relationship should show, if the interpretation be sound, a rectilinear decrease of $K_2 \sin \theta + \sqrt{1 - K_2^2} \cos \theta$ with $\cot \alpha$. The theory of this relationship assumes merely that during geotropic creeping *Tetraopes* turns upward (or downward, in case of positive geotropism) until tension on the legs such as would (unap-

posed) lead to tumbling off the surface is relieved by the attainment of a mechanically stable posture on the inclined plane. It will be brought out subsequently that this simple assumption leads to very interesting results when used to interpret the geotropic orientations of certain other forms (*e.g.*, *Uca*, *cf.* Kropp and Crozier, 1928–29) and of positively geotropic creeping. (Qualitatively, the fact was demonstrated with *Tetraopes* that *similar* changes of θ with α appear when the animal creeps on the *under* surface of a board (*cf.* also Wolf, 1926–27).)

FIG. 10. Diagram of the conditions of oriented progression in *Tetraopes* with abdomen amputated.

With *Tetraopes* however, an additional feature must be considered. The downward slewing of the abdomen (Fig. 7) causes θ to be less, at a given magnitude of α, than if the axis of the body were rigidly perpendicular to the effective "axis" of the legs. This prevents obtaining a good fit of the observed θ's by means of the foregoing equation at low values of α. It is *as if* the animal responded to the requirement of stability not merely by the upward orientation but also by turning the abdomen to one side, thus broadening on that side the base of support. A shifting of the center of gravity, by attaching bits of wax, should in the case of a backward shift cause θ to increase somewhat, as is obvious from Fig. 7, and this in spite of the inclination of the beetle's axis to the plane. A forward shift should cause the limiting value of θ to decrease, for similar reasons, since the base of support broadens up to the points of application of the legs to the substratum. These effects have already been recorded. A further difference appears at quite high values of α, since then the support at the tip of the abdomen becomes inappreciable. The simplicity of the satisfactorily direct proportionality between θ and sin α (Fig. 6) therefore appears to cloak a variety of complications. In the extreme case, when the abdomen is removed, the situation is somewhat altered. We then

conceive the base of support (at low inclinations of the surface) to be limited by a line connecting the points of opposition of the legs on the "down" side. At higher values of α, this condition cannot be met, and orientation must be due to the adjustment of sensibly equivalent tensions on the legs of the two sides. As indicated in connection with Fig. 10, the conditions are in fact too complex for calculation; a more significant test is indeed obtainable by measuring geotropic orientation of beetles of other species, and of ants, in which the abdomen is normally not directly in contact with the inclined substratum.

SUMMARY.

The creeping of the beetle *Tetraopes tetraopthalmus* during negatively geotropic orientation shows the angles of orientation (θ) on a surface inclined at $\alpha°$ to the horizontal to be proportional to $\sin \alpha$. The direction of orientation easily suffers temporary reversal to positive as result of handling. Mechanical stability during upward progression should be just possible when $K_1 \cot \alpha = K_2 \sin \theta + K_3 \cos \theta$, the weight of the body being supported on the tripod formed by the legs on either side and by the posterior tip of the abdomen. Lack of this stability produces tensions on the legs through (1) the bilaterally distributed pull of the body mass on the legs, and (2) the torque on the legs due to the weight of the abdomen. The downward gravitational displacement of the tip of the abdomen causes K_2 and K_3 in the preceding formula to be functions of α.

These relations have been tested in detail by shifting the location of the center of gravity, by attaching additional masses anteriorly and posteriorly, and by decreasing the total load through amputation of the abdomen; the latter operation changes the conditions for stability.

Different formulæ are thus obtained (*cf.* earlier papers) for the orientation of animals in which the mechanics of progression and the method of support of the body weight on an inclined surface are not the same. This demonstrates in a direct way that the respective empirical equations cannot be regarded as accidents. The results are in essence the same as that already obtained with young mammals. The diversity of equations required for the physically unlike cases merely strengthens the conception of geotropic orientation as limited

by the tensions applied to the musculature of the body (caterpillars, slugs) or of appendages (beetles, and certain other forms) when the body is supported upon an inclined surface, since equations respectively pertaining to the several instances, and satisfactorily describing the observations, are deduced on this basis.

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SALT ERROR OF INDICATORS DUE TO STANDARD ALKALINE BUFFERS THEMSELVES. II.

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(From the Chemical Laboratories of Stanford University, California.)

(Accepted for publication, February 4, 1929.)

In a previous paper[1] it was reported that there is a big discrepancy between the true alkalinities or pH values of solutions of standard alkaline buffers and of pure sodium hydroxide which give the same actual color with indicator. The discrepancy amounted to a 100 fold error in the determination of the alkalinity in the most extreme case and was very pronounced with numerous other indicators. Evidently there is a salt error caused by the buffer itself.

Communications from Dr. Mansfield Clark raised the following points: the possible extent of the neutralisation of sodium hydroxide by the added indicator, especially if this had not previously been neutralised, and secondly the practicability of the use of pure dilute solutions of sodium hydroxide as a standard.

The purpose of this communication is to present further experimental work in which these suggested errors have been evaluated. It is found that the former investigation is fully confirmed and even with the rigorous technique the large discrepancies are substantially unaltered.

The colorimetric method previously described has been repeated and extended to include determinations with fully neutralised indicators, direct comparison of neutralised and unneutralised indicator being made with the same standard. The concentration of the hydroxyl ion in the solutions of the pure sodium hydroxide used have been checked by the electrometric method and thus their true alkalinity established in the presence of the indicator. The solutions were prepared as before entirely free from carbon dioxide and carbo-

[1] McBain, J. W., Dubois, O. E., and Hay, K. G., *J. Gen. Physiol.*, 1926, ix, 451.

nate, by the use of sodium drippings,[2] and boiled out conductivity water (1.06×10^{-6}). They were used as soon as prepared and then discarded. In addition for most of the E.M.F. work, oxygen was removed from the water by bubbling with hydrogen or the water was allowed to cool after boiling in an oxygen-free atmosphere. With these precautions higher and far more consistent values have been obtained with the hydrogen electrode than have hitherto been recorded since previous investigators obviously failed with dilute solutions of alkali.

Experimental Method and Materials.

The original method was employed; namely, the direct comparison of colors given by indicators in buffer solutions and those given in pure sodium hydroxide solutions of known concentration and known alkalinity. The buffers used were Sörensen's glycine/sodium hydroxide and Palitzsch's borax/boric acid made according to directions of Clark.[3]

The glycocoll, sodium chloride, borax and boric acid were Kahlbaum's purest. The sodium hydroxide solutions were standardised by Bureau of Standards benzoic acid and constant boiling hydrochloric acid (method of Hulett and Bonner[4]).

The indicators used were:

Thymol violet pH = 9.0 – 13.0, B.D.H. (British Drug Houses).

o-Cresol phthalein, pH = 8.1 – 8.9, B.D.H.

Alizarin yellow G. (*p*-nitrobenzeneazosalicylic acid = alizarin yellow), pH = 10–12.1, B.D.H.

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[2] The method we have used for the past 18 years at Bristol University in connection with precision work on soaps is to place clean metallic sodium in a cone of nickel gauze suspended in a desiccator over a nickel crucible. The desiccator contains a solution of sodium hydroxide free from carbon dioxide. It is essential not merely to use a vacuum desiccator but to have it fitted with well vaselined ground glass stopper to which is sealed on a U-tube containing a strong aqueous solution of hydroxide filling the bend of the U-tube. In this way it is possible to avoid any ingress of carbon dioxide. Solutions prepared in this way exhibited a conductivity 1 per cent greater than the best obtained by other investigators such as the collaborators of W. A. Noyes in 1907, see *Z. physik. Chem.*, 1911, lxxvi, 179.

[3] Clark, W. M., The determination of hydrogen ions, Baltimore, 2nd edition, 1922, 111, 115.

[4] Hulett, G. A., and Bonner, W. D., *J. Am. Chem. Soc.*, 1909, xxxi, 3907.

Tropæolin O (*p*-benzeneazoresorcinsulfonic acid), pH = 11.1 – 12.7 B.D.H.
Thymol blue, B.D.H. (Thymolsulfonphthalein).
Phenolphthalein, "Kahlbaum."
Phenol red, B.D.H. (Phenolsulfonphthalein).

On calculating the effect of the carbon dioxide in the laboratory it is found that there is enough present in 50 cc. of air to neutralise one half of the same volume of 0.0001N sodium hydroxide and all of 0.00001N. Hence special care was taken in making the solutions,

TABLE I.

Concentrations of Indicators Used.

Indicator	Concentration of solution	Amount used per 100 cc. solution
		drops
Alizarin yellow G.	0.01 per cent in water	10
Universal indicator		10
o-Cresol phthalein	0.02 per cent in alcohol	10
	The same neutralised	10
Thymol blue	0.04 per cent monosodium salt in water	10
	0.04 per cent of indicator in water	10
Phenolphthalein	0.5 per cent in 95 per cent alcohol	6
	The same neutralised	6
	0.1 per cent in 50 per cent alcohol	6
Tropæolin O	0.02 per cent in water	10
Thymol violet		3
Phenol red	0.04 per cent monosodium salt	10
	0.02 per cent monosodium salt	10
	0.02 per cent indicator in water	10

using long necked volumetric flasks as containers and running only one sample from the flask. The samples were quickly transferred to Jena test-tubes of 5/8 inch bore and paraffined corks inserted. The matching is done under a daylight lamp.

The results for the buffer solutions are calculated from the formula $pH = \log 1/H^{\cdot} = \log OH' \log 1/K_w$. The pH values of the sodium hydroxide are calculated directly from the known normality, assuming these dilute solutions to be wholly dissociated.

The values of K_w used were:

$$K_w \ 18°C. = 0.72 \times 10^{-14}$$
$$K_w \ 20°C. = 0.80 \times 10^{-14}$$

If the calculations are made from the mean K_w of the values recorded by Kolthoff[5] namely, 0.63 at 18°, and 0.71 at 20° the discrepancies here recorded are enhanced. The experimental results of the colorimetric determinations are recorded in Tables I–VII (work by O. E. C.).

TABLE II.

Concentrations and Hence pH of Sodium Hydroxide Solutions Here Found to Give the Same Color As the Standard Sörensen Glycine Buffers at 18°C., Confirming the Observations of McBain, Dubois and Hay (Bristol) Which Are Put First for Each Indicator for Comparison.

Buffer			Sodium hydroxide with					
Composition		Accepted values	Thymol violet		Tropæolin O		Alizarin yellow	
Glycine	NaOH		Bristol	Clark	Bristol	Clark	Bristol	Clark
2	8	C_{OH} 0.051 pH 12.86	0.046 12.82	0.047 12.82				
3	7	C_{OH} 0.034 pH 12.67		0.032 12.65	0.039 12.74	0.035 12.69		
4	6	C_{OH} 0.018 pH 12.40	0.017 12.50	0.021 12.47	0.021 12.47	0.021 12.47		
4.5	5.5	C_{OH} 0.0073 pH 12.10	0.011 12.18	0.011 12.18	0.011 12.18	0.010 12.14	0.0096 12.12	0.010 12.14
4.9	5.1	C_{OH} 0.0027 pH 11.57	0.0058 11.89	0.0042 11.76			0.0065 11.96	0.005 11.84
5	5	C_{OH} pH 11.31						0.004 11.74

Phenolphthalein and *p*-cresol phthalein were dissolved as specified, then titrated with 0.1N sodium hydroxide till distinctly colored. This was used as the neutralised indicator. Thymol blue and phenol red were made up by dissolving the pure dye in water, and also by neutralising with one equivalent of sodium hydroxide according to Clark's directions.[6] It would seem inadvisable to grind the sodium

[5] Kolthoff, I. M. and Furman, N. H., Indicators, New York, 1926, 248.

[6] Clark, W. M., The determination of hydrogen ions, Baltimore, 1922, 81.

hydroxide and indicator in the air in the mortar because carbon dioxide is taken up so rapidly. Hence, in addition to following Clark, direct neutralisation of indicator was also made.

It will be seen from Table II that the readings in this laboratory closely parallel the readings in the previously published paper in matching thymol violet, alizarin yellow G and tropæolin O, thus in-

TABLE III.

Buffer		Buffer 18°	Thymol blue Bristol 18°	Buffer 20°	Thymol blue (Clark)	
Glycine	NaOH				Neutral	Not neutral
8	2	C_{OH} 0.000016	0.000099	0.000016	0.000082	0.00012
		pH 9.36	10.14	9.31	10.01	10.18

Salt error found in the Bristol paper 10.14 − 9.36 = 0.78 pH.
" " " " this laboratory using indicator 1 equivalent NaOH 10.01 − 9.31 = 0.70 pH.
Salt error using indicator dissolved in water 10.18 − 9.31 = 0.83 pH.

TABLE IV.

Buffer		Buffer 18°	o-Cresol phthalein	Buffer 20°	o-Cresol phthalein (Clark)	
Glycine	NaOH				Neutral	Not neutral
7.5	2.5	C_{OH} 0.000036		0.00024	0.000098	0.000099
		pH 9.66		9.48	10.090	10.092
			Bristol			
8	2	C_{OH} 0.000016	0.000099	0.000016	0.000078	0.000079
		pH 9.36	10.14	9.31	9.99	9.994

Salt error found in Bristol paper 10.14 − 9.36 = 0.78 pH.
" " " " this laboratory 9.99 − 9.31 = 0.68 pH.

dicating that the results are easily reproducible if pure solutions of sodium hydroxide are employed.

It is found that the discrepancy varies according to the manner of preparing the solution of indicator, but that the variation is unimportant compared with the total difference between the pH of the buffer and the pH of the sodium hydroxide solution giving any one color with the indicator. Such a discrepancy of say 0.7 units of pH is an error of fivefold in the measurement of the concentration of hy-

droxyl ion by the usual method of employing these indicators and buffers.

There is no appreciable difference between the results when using *o*-cresol phthalein as such or the same solution titrated with sodium hydroxide until distinctly colored.

In the case of Tables V and VI the figures quoted from the Bristol paper should be revised, especially since the new results were confirmed

TABLE V.

Sörensen buffer. Boric acid.		Buffer 18°	Phenolphthalein 0.5 per cent			Phenolphthalein in 1 per cent
Borax	NaCl		Bristol	Neutral	Not neutral. Clark	Neutral
10	0	C_{OH} 0.000012 pH 9.24	0.00058 10.91	0.0003 10.62	0.0003 10.62	0.000092 10.11
9	1	C_{OH} 0.0000092 pH 9.11	0.0005 10.84	0.00027 10.57	0.00027 10.57	
8	2	C_{OH} 0.0000065 pH 8.98	0.00038 10.72	0.00022 10.48	0.00023 10.50	
7	3	C_{OH} 0.0000051 pH 8.84	0.00033 10.66	0.00017 10.37	0.00018 10.40	
6	4	C_{OH} 0.0000036 pH 8.69	0.000285 10.59	0.00013 10.25	0.00014 10.29	

Salt error found in Bristol paper 10.91 − 9.24 = 1.67 pH (0.5 per cent).
" " " " this laboratory 10.62 − 9.24 = 1.38 pH (0.5 per cent).
" " " " " " 10.11 − 9.24 = 0.87 pH (0.1 per cent).

by using borax, half of which had first been washed away, and by using recrystallised borax, and by obtaining a sample of the borax used at Bristol, and by varying the amount of phenolphthalein, and finally by comparing the two original samples of borax electrometrically.

The colorimetric work may be summarised as follows; the serious difference between the alkalinity of the standard buffers and the concentration of pure sodium hydroxide giving the same color upon addition of indicator is confirmed. The differences between buffer

and pure alkali depend upon the indicator and its concentration, being less for less indicator. Neutralising *o*-cresol phthalein and phenolphthalein had little effect but in the case of thymol blue and phenol red neutralisation does make a small difference. The difference in the

TABLE VI.

Buffer. Boric acid		Buffer 18°	Thymol blue. Bristol	Thymol blue. Clark	
Borax	NaCl			Neutral	Not neutral
10	0	C_{OH} 0.000012 pH 9.24	0.000296 10.61	0.000076 10.02	0.000098 10.16
9	1	C_{OH} 0.0000062 pH 9.11	0.000197 10.44	0.000065 9.95	
8	2	C_{OH} 0.0000065 pH 8.98	0.000148 10.31	0.000052 9.86	
7	3	C_{OH} 0.0000051 pH 8.84		0.000042 9.77	
6	4	C_{OH} 0.0000036 pH 8.69		0.000033 9.66	
5.5	4.5	C_{OH} 0.0000029 pH 8.60	0.000099 10.14	0.00003 9.62	
5	5	C_{OH} 0.0000018 pH 8.41			
10 to 25 drops were used.					

Salt error found in Bristol paper 10.61 − 9.24 = 1.37 pH.
" " " " this laboratory 10.02 − 9.24 = 0.78 pH*
" " " " " " 10.16 − 9.24 = 0.92 pH.†

* Indicator plus 1 equivalent NaOH.
† Pure indicator.

alkalinity of the buffer and the concentration of sodium hydroxide of the same color, is shown in graphical form in the broken lines in Fig. 1. As will be shown in the following section dealing with the electrometric measurements most of the discrepancy must be attributed to salt

error caused by the alkaline buffers themselves. This is represented by the solid lines in Fig. 1 (see later). Between pH's 12 and 13 with glycine buffers, alizarin yellow G, thymol violet and tropæolin O are free from it but at pH 9.4 it amounts to about 0.6 pH and 0.8 pH units

TABLE VII.

Buffer. Borax	Buffer. Boric acid NaCl	Buffer 18*	Phenol red. Bristol	Phenol red (Clark) 0.04 per cent. Neutral	Phenol red (Clark) 0.02 per cent Neutral	Phenol red (Clark) 0.02 per cent Not neutral
3	7	C_{OH} 0.00000087 pH 8.08	0.000124 10.24	0.000097 10.13	0.000062 9.92	
2.5	7.5	C_{OH} 0.0000006 pH 7.94		0.000090 10.12	0.000058 9.91	
2.3	7.7	C_{OH} 0.00000055 pH 7.88	0.000099 10.14	0.000093 10.11	0.000055 9.88	
2	8	C_{OH} 0.00000042 pH 7.78		0.000085 10.07	0.000051 9.85	0.00015 10.33
1.5	8.5	C_{OH} 0.00000029 pH 7.60	0.000046 9.80		0.000046 9.81	
1.0	0.9	C_{OH} 0.00000016 pH 7.36	0.000025 9.54	0.000055 9.88	0.000038 9.72	

Salt error found in Bristol paper 9.80 − 7.60 = 2.20 pH.
" " " " this laboratory 10.07 − 7.78 = 2.29 pH.*
" " " " " " 9.85 − 7.78 = 2.07 pH.†
" " " " " " 10.33 − 7.78 = 2.55 pH.‡

* Phenol red (0.04 per cent) plus NaOH.
† " " (0.02 per cent) plus NaOH.
‡ " " (pure indicator).

for *o*-cresol phthalein and thymol blue respectively. The worst case occurs with borate buffers at pH 7.3 with phenol red where the discrepancy is 2½ pH units. In general the error is negligible in strong alkali such as pH 13 and increases steadily and rapidly as the alkalinity diminishes.

Part II. Electrometric Determinations.

Proof that the colorimetric work is not illusory is obtained by electrometric determinations of the actual alkalinity of the pure sodium hydroxide itself, before and after adding the indicators.

Leeds and Northrup's best standard equipment was used, employing a Clark's cell, a 0.1N calomel electrode and a saturated potassium

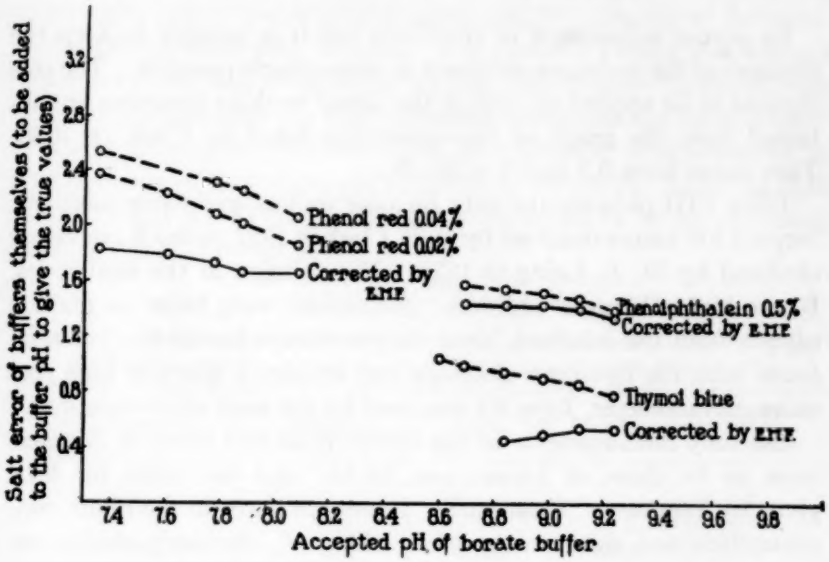

FIG. 1. Comparison between the pH values of standard borate buffers and the concentration and true alkalinity of aqueous sodium hydroxide which give the same color with an indicator. The broken lines show how much greater (in pH units) is the concentration of sodium hydroxide used above that of the hydroxyl ion in the buffer; the solid lines show the difference in true pH (corrected for any action of the indicator upon the dilute sodium hydroxide); that is, the salt error caused by the alkaline buffer itself.

chloride salt bridge and special care was taken to avoid contamination of the sodium hydroxide by the potassium chloride. A number of calomel electrodes were used in each measurement as a check and measurements were not recorded where there was a difference of a few tenths of a millivolt.

The cell measured was:

(Pt.)	H_2 , NaOH	KCl	KCl, Hg_2Cl_2	Hg
	1 atm. 0.1–0.0001	saturated	0.1 solid	

The pH was calculated from the following equations:

$$\frac{\text{E.M.F. (observed)} - \text{Eh (calculated electrode)}}{0.00019837\ T} = \log 1/H^{\cdot} = \text{pH, Clark, p. 158}$$

Corrected for pressure

$$\frac{\text{E.M.F} + \text{E (bar)} - \text{Eh (calculated)}}{0.00019837\ T} = \log 1/H^{\cdot} = \text{pH, Clark, p. 161.}$$

By proper adjustment of the Clark cell it is possible to keep the pressure at the hydrogen electrode at atmospheric pressure. The corrections to be applied to each of the actual working pressures are obtained from the graph of the corrections listed by Clark (p. 409). They range from 0.2 to 0.4 millivolts.

Table VIII presents the data for pure sodium hydroxide solutions. Series A are values obtained by O. E. Clark in 1927; series B are values obtained by M. E. Laing in 1928. The voltages in the final series, B, are higher because additional precautions were taken to exclude oxygen from the solutions, since oxygen always lowers the potential found with the hydrogen electrode and because a specially high sensitive galvanometer, Type HS was used for the most dilute solutions.

The only measurements in the literature of this series of dilutions seem to be those of Lorenz and Mohn[7] and the value for 0.1N given by Sörensen. These earlier values are lower in the dilute concentrations and show an excessive falling off, obviously due to the neutralisation of their sodium hydroxide by the carbon dioxide of the air.

The values were -1.096, -1.045, -0.951 at 30° and 0.1* -1.0891 at 18°.

TABLE VIII.

E. M. F. Data for Solutions of Sodium Hydroxide.

	Series A		Series B	
Concentration	E.M.F.	pH	E.M.F.	pH
0.1	1.094	12.85	1.0965	13.0
0.01	1.041	12.06	1.0435	12.14
0.001	0.9807	10.94	0.9876	11.18
0.0001	0.9156	9.91	0.9230	10.07

[7] Lorenz, R., and Mohn, A., *Z. physik. Chem.*, 1907, lx, 422.

* Sörensen, S. P. L., *Biochem, Z.*, 1909, xxi, 131.

The E.M.F. of a concentration cell involving two solutions of sodium hydroxide with no diffusion is $\frac{RT}{F} \ln c_1/c_2$ and the change in E.M.F. at the working temperature 23° should be 58.7 millivolts and at 20° 58.1 for a tenfold drop in hydroxyl ion if dissociation is complete. In series (*a*) the change in millivolts found for successive tenfold dilutions are 53, 60 and 64 and the final series (*b*) 53, 56 and 65. This means substantial accuracy in spite of the fact that the difficulty of the measurements increases with dilution. For example Lorenz and Mohn's value for 0.001 had dropped by no less than 94 millivolts from that for 0.01 and ours are the only successful measurements for N/10,000.

The results of the corresponding electrometric determinations in the presence of indicators are recorded in Tables IX–XII. In both series (*a* and *b*), 10 drops of indicator are added to 10 cc. of freshly diluted sodium hydroxide.

TABLE IX.

pH Determinations of Pure Aqueous Sodium Hydroxide Alone Compared with Similar Solutions Containing Neutralised and Unneutralised Thymol Blue.

Concentration of solution	Temperature	E.M.F.	pH	Reduction in pH due to indicator
		Series (a)		
	°C.			
0.001 NaOH	23	0.9800	10.94	
0.001 NaOH + neutralised T.B.	23	0.9725	10.82	0.13
0.001 NaOH + unneutralised T.B.	23	0.9716	10.80	0.14
0.0001 NaOH	22	0.9131	9.34	0.30
0.0001 NaOH + neutralised T.B.	22	0.8970	9.53	
0.0001 NaOH + unneutralised T.B.	22	0.8881	9.41	0.42
		Final series (b)		
0.01 NaOH	20	1.0435	12.14	0.04
0.01 NaOH + neutralised T.B.	20	1.0409	12.10	
0.001 NaOH	20	0.9876	11.18	0.04
0.001 NaOH + neutralised T.B.	20	0.9850	11.14	
0.0001 NaOH	20	0.9230	10.07	0.19
0.0001 NaOH + neutralised T.B.	20	0.9106	9.88	

The neutralised indicators of series (*a*) were kept in stoppered flasks. They were likewise used in work preparatory to the final series (*b*). The pH difference between pure 0.01 and 0.001N sodium hydroxide on the one hand and these solutions containing thymol blue on the other were −0.09 and −0.15 respectively, giving practically the same result as in series (*a*).

It should be noted that the pH differences of final series (*b*) are half these of series (*a*) due to improved technique in handling these dilute solutions.

Table X records final series (*b*) with neutralised and unneutralised 0.02 per cent phenol red indicator.

TABLE X.

Effect of Neutralised and Unneutralised Phenol Red upon Solutions of Sodium Hydroxide.

Concentration of solution	Temperature	E.M.F.	pH	Lowering of pH due to indicator
		*Final series (b)**		
	°C.			
0.0026 NaOH	20	1.0090	11.55	
0.0026 NaOH + neutralised P.R.		1.0077	11.52	0.02
0.001 NaOH	18	0.9854	11.22	
0.001 NaOH + neutralised P.R.		0.9845	11.20	0.02
0.0003 NaOH	20	0.9566	10.65	
0.0003 NaOH + neutralized P.R.		0.9438	10.43	0.22

* Fresh 0.0001 NaOH neutralised P. R. as used in series (*a*) gave pH difference of 0.20.

Table XI contains the data for solutions of sodium hydroxide with and without neutralised and unneutralised phenolphthalein.

The indicator is made with 0.10 gm., of Kahlbaum's solid indicator in 50 cc. each of water and alcohol, both being boiled out immediately before use. Two procedures were used in neutralising the phenolphthalein (not following Clark). Either the phenolphthalein is titrated direct with 0.1N sodium hydroxide until distinctly pink or the

calculated amount of solution of sodium hydroxide required to make the monosodium salt is added.

TABLE XI.

Effect of Neutralised and Unneutralised Phenolphthalein upon Solutions of Sodium Hydroxide.

Concentration of solution	Temperature.	E.M.F.	pH	Lowering of pH due to indicator
		Series (a)		
	°C.			
0.001 NaOH	24	0.9807	11.01	
0.001 NaOH + neutralised Ph.		0.9787	10.99	0.02
0.001 NaOH + unneutralised Ph.		0.9770	10.96	0.05
0.0001 NaOH	22	0.9140	10.91	
0.0001 NaOH + neutralised Ph.		0.8874	9.46	0.45
0.0001 NaOH + unneutralised Ph.		0.8868	9.45	0.46
		*Final series (b)**		
0.01 NaOH	19	1.0406	12.13	0.01
0.01 NaOH + neutralised Ph.		1.0396	12.12	
0.0026 NaOH	20	1.0090	11.55	0.02
0.0026 NaOH + neutralised Ph.		1.0076	11.53	
0.001 NaOH	18	0.9857	11.22	0.02
0.001 NaOH + mono Na Ph.		0.9845	11.20	
0.0003 NaOH	20	0.9565	10.65	0.24
0.0003 NaOH + neutralised Ph.		0.9427	10.41	
0.0001 NaOH	19	0.9227	10.10	0.20
0.0001 NaOH + neutralised Ph.		0.9114	9.90	

* Other results preparatory to series (*b*) for 0.001 NaOH plus sodium salt pH difference = 0.03; for 0.0026 NaOH plus 2 drops 0.5 per cent indicator pH difference = 0.002.

DISCUSSION.

Granted that we have sufficiently shown that we have succeeded in preparing dilute solutions of sodium hydroxide of substantial purity

TABLE XII.

Data Using Alizarin Yellow G and 0.05 Molar Borax.

Concentration of solution	Temperature	E.M.F.	pH	Difference in pH
	°C.			
0.001 NaOH	18	0.9854	11.22	
0.001 NaOH + alizarin yellow G		0.9825	11.17	−0.05
0.0001 NaOH	18	0.9227	10.10	
0.0001 NaOH + alizarin yellow G		0.9093	9.90	−0.20
0.05 borax	19.8	0.8757	9.25	
0.05 borax		0.8764	9.27	+0.02

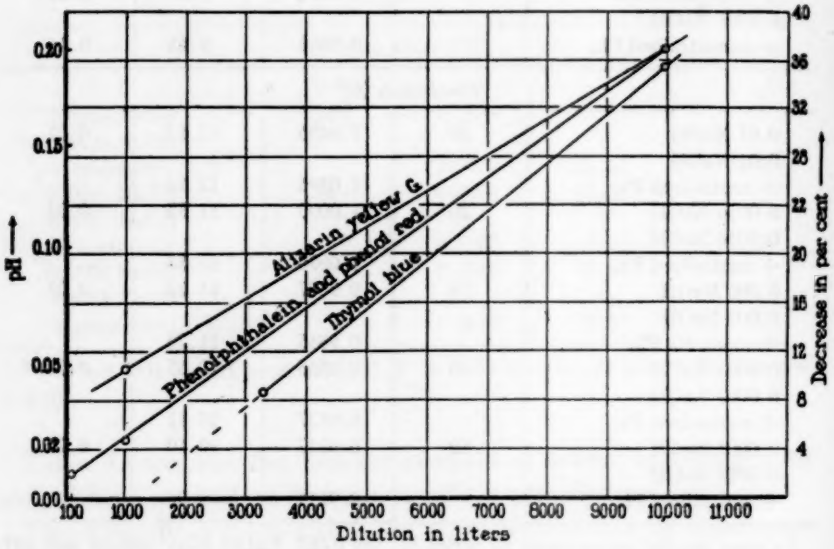

FIG. 2. Correction diagram showing extent to which the true pH of pure dilute aqueous sodium hydroxide is lowered by the addition of indicators.

the main conclusion of our colorimetric work stands; namely, that there is a large salt error inherent in the standard buffers themselves.

It is true that there is a slight neutralising effect of the indicators but these are such weak acids and their salts are so extensively hydrolysed as to make this a distinctly minor effect not exceeding 0.2 pH in the most dilute solutions used. It is therefore usually much less than the error of colorimetric determinations (0.2 pH corresponds to an error of 60 or −37 per cent).

The extent of the relatively slight neutralising action of the four indicators, alizarin yellow G, phenolphthalein, phenol red and thymol blue is shown in Fig. 2 for various dilutions (in liters) of pure sodium hydroxide. The neutralisation diminishes the alkalinity of the sodium hydroxide approximately proportional to the dilution. Two scales are provided in Fig. 2 so that the effect on the indicator in diminishing the true alkalinity of the sodium hydroxide as determined by the hydrogen electrode may be read either in pH units or in per cent decrease in concentration.

It will be seen that all four indicators produce almost the same effect in N/10,000 sodium hydroxide, namely 0.2 pH units decrease of alkalinity but that for phenolphthalein and phenol red this has fallen to the negligible value of 0.02 pH units for N/1000 sodium hydroxide being still less for thymol blue and even for alizarin yellow G being only 0.05 pH units.

These corrections are negligible in comparison with the serious salt errors found, as can be seen from Fig. 1, where the broken lines recorded the actual concentration of hydroxide giving the same color as the buffer and the corresponding solid lines are corrected by means of Fig. 2.* For example phenol red still shows as much as two whole pH units salt error, due to the borate buffer, that is, an error of 99 fold.

SUMMARY.

Previous results of the comparison of colors given by indicators in alkaline buffers and pure aqueous sodium hydroxide have been repeated and confirmed. The electrometric determinations show that the sodium hydroxide was pure and gave theoretical values for the

* It may be mentioned that the results in Fig. 2 have been made use of in the calculation of the alkalinity of a comprehensive set of data for soap solutions by McBain and Hay (now communicated to the *J. Chem. Soc.*). Linear extrapolation has been used for dilutions greater than 0.0001 N.

concentration of hydroxyl ion. The slight but distinct neutralising effect of dilute solutions of alkali has been measured electrometrically and the allowances to be made are recorded graphically. It is found that whereas alizarin yellow G, tropæolin O and thymol violet may be used without appreciable error (in accordance with our previous communication) the grave discrepancies remain for phenolphthalein, *o*-cresol phthalein and thymol blue and phenol red which must be ascribed to salt error in the alkaline buffer itself.

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